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THE AMERICAN REVIEW OF TUBERCULOSIS

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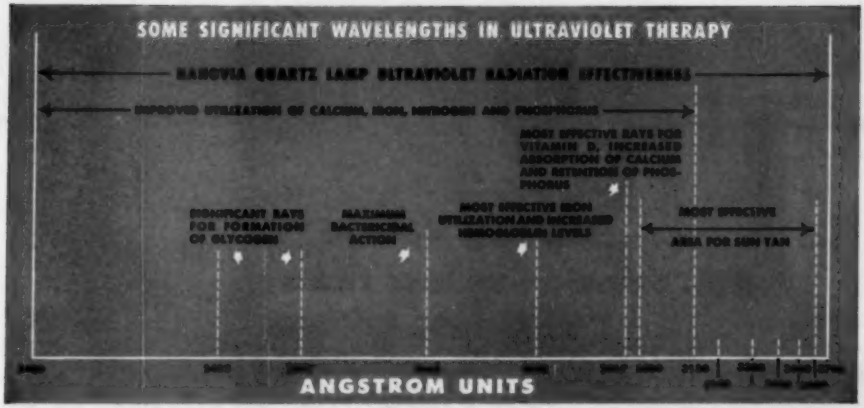
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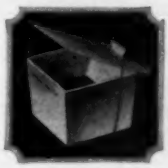

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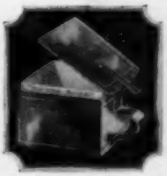

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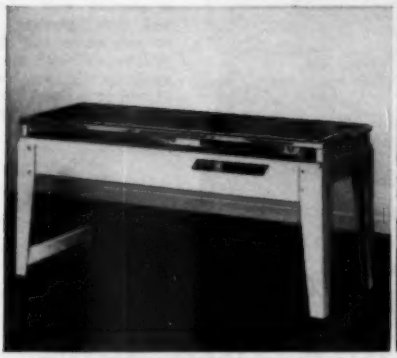

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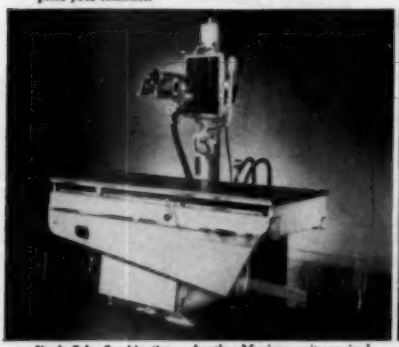

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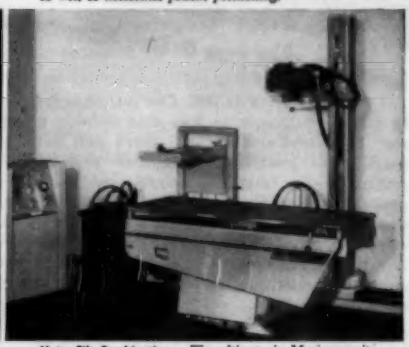

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THE AMERICAN REVIEW
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PLASTIC RECONSTRUCTION OF TRACHEA AND BRONCHI[1,2]

DONALD L. PAULSON

(Received for publication May 17, 1951)

INTRODUCTION

Restoration of the bronchial or tracheal lumen in the interest of preservation of lung tissue or life itself constitutes an intriguing challenge to the thoracic surgeon today. Trauma, tumors, tuberculosis, and other infections may compromise the lumen of these respiratory passages. Reliable reconstructive procedures are desirable to restore function and prolong life without unwarranted sacrifice of lung tissue.

A review of previous experience reveals that any tracheal or bronchial reconstructive procedure must meet certain requirements to be successful. (*1*) An adequate airway through a closed circuit must be maintained during the operation. (*2*) A fairly rigid, noncollapsible wall should be provided to serve as a scaffold for the regeneration of tracheobronchial epithelium. (*3*) An adequate airtight passage which is permanent without eventual stenosis should be obtained. (*4*) Provision should be made for the ready removal of tracheobronchial secretions during the early postoperative period before the regenerating lining epithelium becomes functionally effective in this regard.

Experimental Review

The surprising healing and regenerative powers of the trachea and bronchi have been well demonstrated experimentally. Taffel (29) effected an airtight repair of resected small, full-thickness segments of the trachea and bronchus in dogs by means of free fascial grafts. The grafts underwent connective tissue replacement, forming an outer layer lined by pseudostratified epithelium with a layer of submucosa, mucous glands, and a few small islands of new cartilage. Daniel (4) repeated these experiments and found that some stenosis developed. Using foreign substances, such as glass, stainless steel, or Vitallium tubes, Daniel found that regeneration of the tracheal and bronchial elements including mucosa and cartilage occurred around these rigid tubes with no stenosis. Grindlay, Clagett, and Moersch (10) have used polyethylene tubing for experimental reconstruction of the trachea and have observed regeneration of elements of the tracheal wall except for cartilage, little stenosis, and no troublesome secretions. Rienhoff, Gannon, and Sher-

[1] From the Southwestern Medical College of the University of Texas, Dallas, Texas.

[2] Presented before the Medical Session, as part of *Surgery*, at the 47th annual meeting of the National Tuberculosis Association, Cincinnati, Ohio, May 17, 1951.

man (26) have shown that satisfactory healing of the cut end of a bronchus may be achieved in the dog simply by airtight approximation of pleura. Hanlon (11) has achieved the same result with gelatin sponge.

Complete healing without stenosis occurs in dogs after division or resection of segments of trachea or bronchus and end-to-end anastomosis (2, 7, 14, 22). The length of tracheal segment which can be removed and still permit an anastomosis varies from 2.5 to 8 cm. depending on the elasticity of the trachea, the mobility of the larynx and carina, and the position of the head and neck. Nowarowski (24) studied human cadavers and found it feasible to resect 3 to 5 cm. of the trachea and still perform an end-to-end suture with the neck in a neutral position. Less length can be removed from a long neck because the annular ligaments are already stretched. Rob and Bateman (27) dissected six fresh human bodies and concluded that end-to-end suture is impossible if more than 2 cm. is removed.

Juvenelle and Citret (17), working with J. D. Stewart, have severed either main bronchus and successfully implanted it 2 or 3 cm. higher by suture to a window in the tracheal wall in dogs. They then resected a portion of the lower trachea and both main bronchi and successfully reconstructed the airway by performing an end-to-end anastomosis of the distal trachea to one of the main bronchi and reimplanting the other main bronchus higher on the trachea.

Homogeneous tracheal transplants fail in the repair of defects in the dog due to absorption and nonreplacement of cartilage, leaving a flaccid, fibrous, epithelial-lined tube which eventually produces stenosis and obstruction (2, 7, 15). There is conflicting evidence concerning autografts. Carter and Strieder (2) have found that autografts of segments of bronchus, from a resected lobe or lung, remain viable for complete healing without stenosis in the repair of large defects in the bronchus or trachea of the dog. On the other hand, Ferguson, Wild, and Wangensteen (7) found marked fibrous stenosis and almost complete disappearance of autografts prepared by resecting a segment from the cervical trachea and immediately suturing it back in place, discarding 2½ rings to permit the use of mucosal cuffs at each end.

The use of tantalum gauze tubes alone to replace the cervical trachea in dogs results in the trachea's being covered by granulation tissue which later develops into a fibrous stricture with no epithelial covering (7).

Clinical Review

End-to-end anastomosis of the trachea and bronchi in human beings has been successfully achieved. Several resections and anastomoses of human trachea have been reported, the first apparently in 1884 by Kuester (18). Segments 2 to 4 cm. in length were removed. Griffith (9) resected a traumatic bronchial stricture and performed a successful end-to-end suture. Tuttle (30) anastomosed a left upper lobe bronchus directly to the left main bronchus following a technically difficult left lower lobectomy. Lester (20) successfully sutured a torn and separated left main bronchus two weeks after the patient was injured.

As a result of experimental observations, tubes made of various foreign substances have been used in the reconstruction of the trachea. Clagett, Grindlay, and Moersch (3) reported the use of a polyethylene tube in one patient in whom the trachea, carina, and right lung were resected for a cylindroma. The patient died on the sixth day as a result of inability to raise secretions and impingement of the tube on the left upper lobe bronchial orifice. Jarvis (16), following the work of Daniel, used a stainless steel tube to replace 4 cm. of trachea resected for a cylindroma. One year later he removed the tube but was forced to replace it within three weeks because of lack of rigidity of the regener-

ated tracheal wall. Holmes (13) used a Vitallium tube to bridge a three-fourth-inch gap in which the resection was done for a traumatic stricture. One month later the patient developed difficulty in raising secretions, probably due to the fact that the mucosa was sutured to the ends of the tube and became redundant at these points. Later, the Vitallium tube was replaced by a stainless steel tube. Longmire (21) used a Lucite tube to replace a cervical trachea which had been accidentally avulsed. At ten and one-half months the unsupported channel was not rigid enough to provide an unobstructed air way.

Fascia and skin have been used for reconstruction of the trachea and bronchi. Belsey (1) resected the entire intrathoracic trachea, except for a narrow strip of the left lateral wall, for an adenoma and effected a satisfactory reconstruction by using a fascial graft laid over a spiral of stainless steel wire, sutured to the ends of the trachea. Rob and Bateman (27) used tantalum gauze covered on both sides with fascia lata to effect a satisfactory reconstruction of 9 cm. of the cervical trachea including the cricoid cartilage, resected for a recurrent carcinoma of the thyroid. They had some difficulty with stridor due to flutter of the free fascial lining of the tantalum tube and lack of airtightness resulting in cervical emphysema.

Harrison (12) removed a traumatic tracheal stricture and placed a Thiersch skin graft over the raw surface, with success. Negus used skin flaps to reconstruct the continuity of the laryngotracheal segment in cases of fistula following irradiation. Swinburne, LeJeune, and Owens (28) and Erich (5) have successfully used thin skin grafts in the plastic repair of laryngotracheal stenosis. Fairchild (6), in 1927, remade a segment of cervical trachea in three stages by means of a skin tube.

To Gebauer (8) belongs the credit for demonstrating the superiority of wire-enforced dermal grafts for plastic reconstruction of the trachea and bronchi. He utilized this method entirely for tuberculous stenosis of these structures but advocated its use for other types of lesions as well. Gebauer reported the use of dermal grafts for stenoses involving the bronchi and trachea in thirteen patients. As a result of this experience he called attention to certain differences between bronchial and tracheal reconstructive procedures.

Of the nine patients having dermal grafts to the bronchus, all survived and the grafts were successful from a technical standpoint. Of four tracheal grafts attempted, two died during the operation due to inability to maintain an airway, one died on the third postoperative day due to a narrowed edematous airway (which might have been relieved by tracheotomy), and the fourth survived. The requirements for success in tracheal reconstructive procedures are more exacting and yet more difficult to attain than those for the bronchus. An adequate airway must be maintained but may be difficult in achieving. A minimal amount of trauma during and after the procedure (such as accompanies tracheal intubation) is highly desirable. Provision, such as tracheotomy, should be made for the ready removal of secretions.

According to Gebauer's experience, wire-enforced dermal grafts are advantageous for tracheobronchial reconstructive procedures because the dermis is readily available (from the patient's back) and it is thick, tough and pliable, and elastic. It holds sutures well and does not tear on being pierced with a needle as does fascia or pleura. It adheres to any surface readily and, for these reasons, it forms a rapid airtight closure along the suture lines. With wire-enforcing, the graft acquires the necessary rigidity for the tracheal or bronchial wall. With the cut surface of the dermis facing the tracheobronchial lumen, an ideal surface, rich in vascular and lymphatic channels, is presented for the ingrowth of the

lining epithelium. Finally, the dermal graft appears to survive as living transplanted tissue, providing a scaffold for the regeneration of epithelium. Gebauer's seventh case was one in which a pneumonectomy was done one year after dermal grafts had been applied to both the trachea and the right bronchus. On careful examination the graft was found to have survived, with little change in its elastic and collagen elements. The sweat glands were diminished in number but did not show the changes of degeneration and replacement reported by Peer and Paddock (25).

OBSERVATIONS

The writer's experience with plastic reconstruction of the bronchus and trachea by means of dermal grafts in 3 patients is presented in detail. The indica-

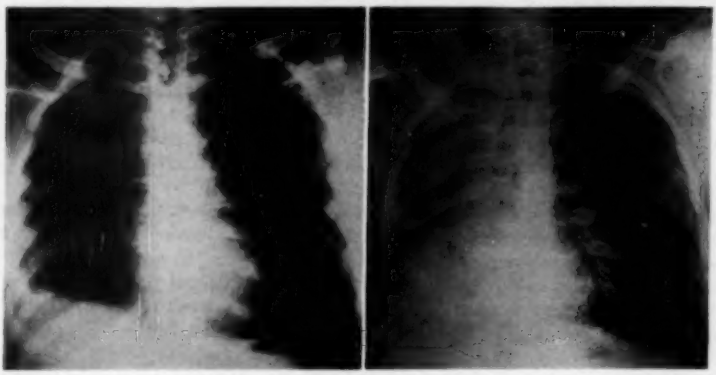

FIG. 1. Case 1. (Left) Roentgenogram illustrating progressive shrinkage of the right lung over a period of seven weeks. (Right) Bronchography illustrated complete occlusion of the right main bronchus at the level of the carina.

tions were a traumatic bronchial occlusion, a tracheal carcinoma, and a tracheal stricture due to tuberculosis.

Case Reports

Case 1. C. M., a 22-year-old oil-field worker, sustained a severe chest injury on May 27, 1949. While unloading heavy drill pipe from a truck, he slipped and fell, the heavy pipe rolling across his chest and neck. A severe tension pneumothorax on the right side and a mediastinal emphysema developed rapidly, necessitating an emergency mediastinotomy and pleural decompression. The lung expanded well, but approximately seven weeks later it became evident that a progressive atelectasis was occurring. Bronchoscopy and bronchography at this time revealed a complete occlusion of the right main bronchus at the level of the carina (figure 1).

It was decided to attempt to save the patient's right lung by means of a plastic reconstruction of the right main bronchus. Accordingly, on July 2, 1949, a thoracotomy was done, anesthetic being administered through an intratracheal

tube inserted into the left main bronchus. The membranous portion of the lower trachea and the right main bronchus was incised, the incision being carried through the area of bronchial occlusion posteriorly. The mucosa was undermined for a short distance and sutured over the heavy, fibrous scar which formed the occlusion. An ovoid defect in the membranous portion of the lower trachea and upper bronchus remained. A dermal graft measuring 2 by 3 cm. was prepared from the skin of the back. The cut surface of the dermis was placed over the tracheobronchial defect and sutured in place by means of fine cotton sutures. Suture of the mediastinal pleura over the graft rendered the new wall airtight to positive pressure.

The patient's postoperative course was uneventful. Early, complete re-expansion of the lung occurred and the patient left the hospital on the seventh postoperative day. The patient was re-examined one month later. Roentgenograms

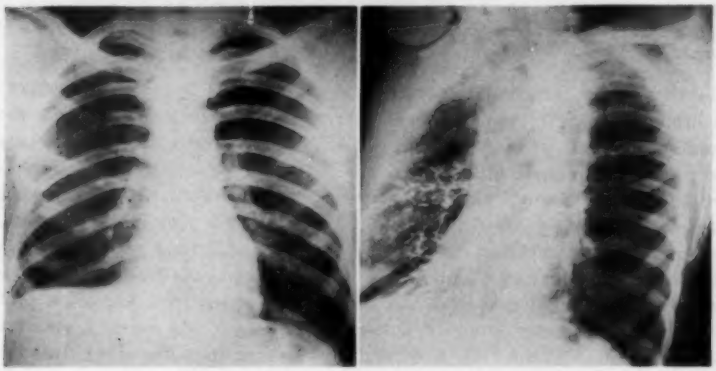

FIG. 2. Case 1. (Left) Complete expansion of the right lung one month following bronchoplasty by means of dermal graft. (Right) Bronchogram at the same time illustrated slight narrowing at the site of the bronchoplasty.

of the chest revealed complete expansion of the right lung, and bronchography revealed a normal bronchial tree with no evidence of dilatation (figure 2). Bronchoscopic examination revealed a patent right main bronchus with an intact normal mucosa. The only evidence of the previous occlusion was a narrowing of the bronchial lumen approximately one-half centimeter distal to the carina. This narrowing is due to the scar in the bronchial wall medially and anteriorly which was not disturbed at the time of the plastic reconstruction. A 7 mm. Broyles bronchoscope could be readily passed through the area of narrowing.

The patient was seen intermittently for one year and has maintained lung expansion, with no evidence of further stenosis. He has had no cough, no wheeze, and no dyspnea, and is working as a laborer.

Comment: A dermal graft was utilized to reconstruct satisfactorily the lumen

of a bronchus occluded by trauma. Preservation of an entire lung in a functional state was thereby achieved. The narrowing of the bronchus produced by traumatic scar is not sufficient to interfere with normal function and has not progressed.

Case 2. W. H., a 56 year-old white male, was first seen in October, 1945, at which time a right pneumonectomy was done for an epidermoid carcinoma involving the right main bronchus and mediastinal lymph nodes. The patient did well with no evidence of recurrence until July 18, 1950, nearly five years later. At this time he complained of coughing up blood for two months, a wheeze, difficulty in raising secretions, and shortness of breath. He had a stridor with evidence of severe respiratory tract obstruction. A bronchoscopic examination revealed a fungating mass arising from the left lateral and anterior walls of the trachea approximately 5 cm. below the larynx and reducing the lumen of the trachea at this point to approximately 3 mm. Nevertheless, a 7 mm. bronchoscope could be readily passed beyond the tumor. The right bronchial stump and left main bronchus appeared normal, the carina was sharp. There were approximately 4 cm. of normal-appearing trachea below the tumor. Biopsies removed from the tumor mass revealed an epidermoid carcinoma. Esophagoscopy and esophagrams revealed a normal esophagus with no evidence of involvement by the carcinoma.

In view of the fact that the tracheal carcinoma was probably secondary to the bronchiogenic carcinoma removed nearly five years before and because there was no other evidence of metastasis, it was decided to resect the tracheal tumor and perform a reconstructive procedure. The patient's attitude of firm opposition to a tracheotomy, which otherwise would be indicated, influenced this decision. Accordingly, on July 25, 1950, resection of a portion of the tracheal wall and reconstruction by means of a wire-enforced dermal graft were accomplished. A low-collar and sternum-splitting incision was made. The trachea and tumor were dissected free from the surrounding structures. A portion of the left tracheal wall, anterolaterally, measuring 3 by 5 cm. was resected, preserving the right posterolateral wall. A dermal graft, taken from the thigh, was laced with No. 32 steel wire and applied to the tracheal defect. It was sutured in place by means of fine cotton sutures in the dermis and tracheal mucosa, re-enforced by a second row of fine cotton sutures between the external surface of the graft and the trachea. During the suture, the graft was carefully molded about the intratracheal tube. The graft appeared to form a fairly rigid wall, to be airtight, and to provide a satisfactory tracheal lumen. A tracheotomy was not done immediately because it was believed that frequent intratracheal aspirations by means of a catheter would be sufficient for the removal of secretions. This did not prove the case, however, and it was necessary to perform an emergency bronchoscopic aspiration on the second postoperative day. A tracheotomy was then done for the more ready removal of secretions. Difficulty in swallowing developed, due to edema of the hypopharynx. All fluids were then administered parenterally. In spite of all measures, evidence of atelectasis in the left lower lobe developed, the patient's temperature gradually rose, and he died on the sixth postoperative day.

Autopsy revealed small focal areas of atelectasis in an emphysematous left lung with small, mucous plugs in the smaller segmental bronchi. No carcinoma

was found in the chest or in any of the organs. The dermal graft was intact and tightly adherent in good position (figure 3).

Comment: In retrospect, a tracheotomy should have been done at the time of the reconstructive procedure. Frequent intratracheal aspirations were not adequate for the removal of secretions. In the two days' time before the tracheotomy was finally done, the patient's one lung was probably already compromised by obstruction of the smaller bronchi. During the ensuing four days the process could not be reversed. Tracheotomy done at the time of the tracheal resection probably would have prevented the chain of events which caused this patient's death.

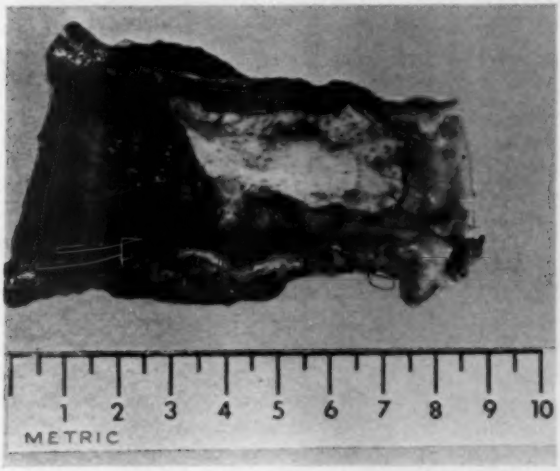

FIG. 3. Case 2. Specimen of trachea removed at necropsy illustrating the dermal graft in position.

Case 3. O. J., a 32-year-old white female, was first seen April 3, 1950. A "spot" on the right lung had been found ten years before. She had noted a cough since 1948. In March 1949, she became worse and developed coughing spasms. Her sputum was found positive for tubercle bacilli. She had rested since June 1949. She continued to cough in paroxysms, to be hoarse, to have a wheeze, shortness of breath, and fever. She had taken streptomycin for eight weeks in 1949.

Roentgenograms of the chest revealed a solid atelectasis of the right upper lobe and infiltration throughout the right lung field. The left lung contained a small lesion at the extreme apex. Bronchoscopic examination revealed a marked granulomatous tracheobronchial tuberculosis beginning approximately 2 cm. below the larynx and extending down the right side of the trachea and completely blocking the right main bronchus. The lumen of the trachea was estimated at 6 mm. in diameter. The patient was instructed to take streptomycin and PAS daily for six weeks and return for re-examination.

Bronchoscopic examination six weeks later revealed complete clearing of the granulomatous lesions in the trachea although there was some narrowing of the lower third. There was a severe stricture of the right main bronchus, the opening being pinpoint in size. A

right pneumonectomy was considered imperative and was done in the prone position on May 20, 1950. The right upper lobe was atelectatic and there were scattered areas of disease throughout the middle and lower lobes. During the immediate postoperative period, frequent intratracheal aspirations were necessary for the removal of secretions. The patient left the hospital on the ninth postoperative day with instructions to continue the streptomycin and PAS daily for another six weeks, as well as an indefinite prolonged period of rest.

The patient was seen again September 5, 1950, at which time she was found to have some stridor but no cough. It was believed that she probably had persistent, and perhaps progressive, tracheal narrowing. She was advised to take streptomycin and PAS for six weeks and to return for a possible dermal graft to the trachea.

FIG. 4. Case 3. Roentgenogram illustrating severe subglottic tracheal stenosis.

The patient did not return until December 20, 1950, at which time she was admitted to the hospital by ambulance because of marked shortness of breath. Approximately one month previously the patient had noted an increase in her stridor and a "bubble in her throat" cleared by a slight cough. Slight exertion produced a feeling of obstruction. Her exercise tolerance progressively diminished so that she was unable to get out of bed. Roentgenograms of the chest and neck revealed displacement of the trachea upward and to the right, slight narrowing as it entered the thorax, and severe subglottic stenosis (figure 4). Using a 3 mm. bronchoscope, the trachea was examined. A tracheal stricture just below the true cords was visualized. As soon as the bronchoscope was passed through the area, the stridor disappeared and the patient breathed comfortably. The stricture was covered by smooth, glistening epithelium. The lower trachea was narrowed as was the left main bronchus, but the size of the lumen appeared adequate. The bronchial stump was well healed and there was no evidence of active tracheobronchial tuberculosis. The mucosa was thin and resembled parchment. A specimen of bronchial secretions was negative for tubercle bacilli on smear and later by culture.

After due consideration of the findings, a plastic procedure on the upper trachea was decided upon as a matter of necessity. On December 27, 1950, a dermal graft was removed from the back and prepared for use. Through a cervical incision, the trachea was dissected free from the hypopharynx and incised posteriorly from the cricoid cartilage to the base of the neck for a distance of 4 cm. The dermal graft was threaded with No. 32 steel wire so that it could be molded in a circular direction and applied to the trachea to bridge the elliptical defect made by the incision of the strictured area. The graft was sutured in place by means of interrupted fine cotton sutures. The intratracheal tube was withdrawn and a tracheotomy performed, using a No. 4 tracheotomy tube.

The patient's postoperative course was stormy due to difficulty in maintaining an airway through the lower trachea and left main bronchus. The secretions were thick, and crusts formed repeatedly over the end of and below the tracheotomy tube. Repeated bronchoscopic aspirations through the tracheotomy opening were necessary until the third postoperative day when the metal tracheotomy tube was replaced by a curved rubber No. 24 F intratracheal tube long enough to enter the left main bronchus. This tube provided a good airway, but crusting over the tip of the tube forced its frequent changing. The rubber tracheotomy tube was removed on the sixth postoperative day. Occasional intratracheal aspirations were necessary until the twelfth postoperative day. The patient's exercise tolerance improved remarkably, so that she was able to walk about the hospital with no dyspnea or stridor. She was discharged from the hospital on the sixteenth postoperative day.

The patient returned one week later complaining of a wheeze on inspiration which had developed one day prior to her return to the hospital. Her exercise tolerance was still excellent. Bronchoscopy done at this time revealed no evidence of a stricture or narrowing in the upper portion of the trachea. The area of the skin graft was perfectly smooth and appeared to be covered by glistening tracheal mucosa. At one point a piece of steel wire could be visualized. The right bronchial stump appeared normal. Beginning at the level of the carina and extending into the left main bronchus there were masses of granulation tissue; beyond this, in the left main bronchus, some gray-tipped granulations were present. This was undoubtedly due to a submucosal tuberculosis which progressed following the loss of the thin mucosa overlying it at the time. Bronchial secretions were taken for bacteriologic study, and the granulations were painted with 30 per cent silver nitrate. Bacteriologic studies were again negative for tubercle bacilli. The patient did well following this bronchoscopy, and her wheeze completely disappeared. She returned home with instructions to return to the hospital in two weeks for another bronchoscopy. The day before her scheduled return, the patient again developed a wheeze and difficulty in raising her bronchial secretions. She began the trip to the hospital immediately, but on the way she suddenly died.

Comment: This patient developed a tracheal stenosis, secondary to a tracheobronchial tuberculosis. A plastic reconstruction of the upper portion of the

trachea by means of a dermal graft was successful. Reactivation of probably submucosal tuberculosis in the left main bronchus produced a bronchial obstruction which accounted for the patient's death. Repeated trauma to this area following the plastic reconstruction of the upper trachea was undoubtedly responsible for reactivation of the disease in this area. Bronchoscopy done a little more than three weeks following the plastic reconstruction of the trachea revealed a satisfactory mucosal lining. The patient had obtained complete relief from the tracheal stridor and her exercise tolerance had returned to normal before the obstruction in the left main bronchus developed.

DISCUSSION

As a result of a review of the experience of others and that obtained from the three cases herein reported, it appears that wire-enforced dermal grafts are satisfactory for plastic reconstruction of the trachea and bronchi. The dermal graft provides a scaffold for the regeneration of epithelium. Enforcement by wire provides enough rigidity to prevent collapse. The tracheobronchial epithelium apparently covers over the dermal graft.

In those cases in which it is possible to resect 2 to 4 cm. of the trachea, an end-to-end anastomosis may be possible. In most instances, however, a more extensive resection will be required. The surgeon then has a choice of the use of a rigid tube, fascia, or dermis in conjunction with either steel wire or tantalum gauze. In those cases in which rigid tubes have been used, difficulty has been encountered because of the lack of rigidity of the regenerated tracheal wall. The most successful cases have been those in which fascia or skin has been used in conjunction with steel wire or tantalum gauze. Of particular importance in this consideration is the fact that by the use of fascia or skin with tantalum gauze or steel wire a rigid tube has been provided and the tracheal epithelium has grown on its inner side. In the case of polyethylene, stainless steel, glass, or Vitallium tubes, the epithelium grows on the outer side. This is probably true because the solid cylinder cannot be penetrated by granulation tissue. That the fascia or dermis presents a scaffold for the ingrown epithelium is well demonstrated by Rob and Bateman's experience in which they used fascia on both sides of the tantalum gauze in reconstruction of a cervical trachea and obtained an ingrowth of tracheal epithelium on the inner surface; whereas in reconstruction of the cervical esophagus they used tantalum gauze with fascia on only the outer surface and obtained growth of esophageal epithelium outside the tantalum gauze.

SUMMARY

A review of the experimental and clinical experience with reconstructive procedures of the trachea and bronchi has been made and three cases in which dermal grafts were used are reported in detail. It is believed that wire-enforced dermal grafts are satisfactory for plastic reconstruction of the trachea and bronchi.

SUMARIO

Reconstrucción Plástica de la Tráquea y los Bronquios

Esta reseña comprende observaciones experimentales y clínicas de procedimientos reconstructivos de la tráquea y los bronquios, presentando además con todo pormenor tres casos en que se usaron injertos dérmicos. Parece que los injertos dérmicos reforzados con alambre son satisfactorios para la reconstrucción plástica de los órganos mencionados.

RESUME

Reconstitution plastique de la trachée et des bronches

Revue des travaux cliniques et expérimentaux sur les procédés de reconstitution plastique de la trachée et des bronches et trois cas dans lesquels des greffons dermiques ont été utilises sont rapportés. L'auteur estime que les greffons dermiques renforces par des fils métalliques sont satisfaisants pour la reconstitution plastique de la trachée et des bronches.

REFERENCES

(1) Belsey, R.: Stainless steel wire suture technique in thoracic surgery, Thorax, 1946, *1*, 39.

(2) Carter, M. G., and Strieder, J. W.: Resection of the trachea and bronchi, J. Thoracic Surg., 1950, *20*, 613.

(3) Clagett, O. T., Grindlay, J. H., and Moersch, H. J.: Resection of the trachea: An experimental study and report of a case, Arch. Surg., 1948, *57*, 253.

(4) Daniel, R. A., Jr.: Regeneration of defects of the trachea and bronchi: An experimental study, J. Thoracic Surg., 1948, *17*, 335.

(5) Erich, J. B.: Treatment of extensive cicatricial stenosis of the larynx or the trachea, Arch. Otolaryng., 1945, *41*, 343.

(6) Fairchild, F. R.: Reconstruction of the trachea, Surg., Gynec. & Obst., 1927, *44*, 119.

(7) Ferguson, D. J., Wild, J. J., and Wangensteen, O. H.: Experimental resection of the trachea, Surgery, 1950, *28*, 597.

(8) Gebauer, P. W.: Plastic reconstruction of tuberculous bronchostenosis with dermal grafts, J. Thoracic Surg., 1950, *19*, 604;
Further experiences with dermal grafts for healed tuberculous stenosis of the bronchi and trachea, J. Thoracic Surg., 1950, *20*, 628;
Experiences with surgical reconstruction of the trachea, Am. Rev. Tubere., 1950, *62*, 176.

(9) Griffith, J. L.: Fracture of the bronchus, Thorax, 1949, *4*, 105.

(10) Grindlay, J. H., Clagett, O. T., and Moersch, H. J.: Resection and anastomosis of the trachea in dog, Ann. Otol., Rhin. & Laryng., 1949, *58*, 1225.

(11) Hanlon, C. R.: Observations on the use of gelatin sponge in closure of experimentally produced defects of the bronchus, Surg., Gynec. & Obst., 1948, *86*, 551.

(12) Harrison, W. J.: Traumatic stenosis of the trachea treated with skin grafting, Brit. M.J., 1936, *2*, 811.

(13) Holmes, C. L.: Discussion of Jackson, O'Brien, Tuttle, and Meyer; Carter and Strieder; and Gebauer: J. Thoracic Surg., 1950, *20*, 650.

(14) Jackson, T. L., Tuttle, W., and O'Brien, E. J.: An experimental study in bronchial anastomosis, J. Thoracic Surg., 1949, *18*, 630.

(15) Jackson, T. L., O'Brien, E. J., Tuttle, W., and Meyer, J.: The experimental use of homogeneous tracheal transplants in the restoration of continuity of the tracheobronchial tree, J. Thoracic Surg., 1950, *20*, 508.

(16) Jarvis, F. J.: Discussion of Jackson, O'Brien, Tuttle, and Meyer; Carter and Strieder; and Gebauer, J. Thoracic Surg., 1950, *20*, 647.
(17) Juvenelle, A., and Citret, C.: Discussion of Jackson, O'Brien, Tuttle, and Meyer; Carter and Strieder; and Gebauer, J. Thoracic Surg., 1950, *20*, 647.
(18) Kuester, K. F.: (Quoted by Colley, F.): Die Resektion der Trachea, Deutsche Ztschr. f. Chir., 1895, *40*, 150.
(19) LeJeune, F., and Owens, N.: Chronic laryngeal stenosis, Ann. Otol., Rhin & Laryng., 1935, *44*, 354.
(20) Lester, R. T.: Personal communication, 1950.
(21) Longmire, W. P.: The repair of large defects of the trachea, Ann. Otol., Rhin. & Laryng., 1948, *57*, 875.
(22) Maisel, B., and Dingwall, J. A.: Primary suture of the divided cervical trachea, Surgery, 1950, *27*, 726.
(23) Negus, V. E.: Tracheal stenosis, Ann. Otol., Rhin. & Laryng., 1938, *47*, 891.
(24) Nowarowski, K.: Beitrag zur tracheoplastik, Arch. f. klin. Chir., 1909, *90*, 847.
(25) Peer, L. A., and Paddock, R.: Histologic studies on the fate of deeply implanted dermal grafts: Observations on sections of implants buried from one week to one year, Arch. Surg., 1937, *34*, 268.
(26) Rienhoff, W. E., Jr., Gannon, J., Jr., and Sherman, I.: Symposium of thoracic surgery: Closure of bronchus following total pneumonectomy, Ann. Surg., 1942, *116*, 481.
(27) Rob, C. G., and Bateman, G. H.: Reconstruction of the trachea and cervical oesophagus, Brit. J. Surg., 1949, *37*, 202.
(28) Swinburne, G.: Chronic laryngeal and tracheal stenosis, Australian & New Zealand J. Surg., 1941, *10*, 282.
(29) Taffel, M.: The repair of tracheal and bronchial defects with free fascia grafts, Surgery, 1940, *8*, 56.
(30) Tuttle, W.: Discussion of Jackson, T. L., Lefkin, P., Tuttle, W., and Hampton, F.: An experimental study in bronchial anastomosis, J. Thoracic Surg., 1949, *18*, 641.

A HISTOLOGIC STUDY OF THE BLOOD VESSELS IN SURGICALLY RESECTED TUBERCULOUS LUNGS[1]

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INTRODUCTION

Routine preoperative cardiac catheterization of tuberculous patients selected for lobectomy or pneumonectomy has demonstrated elevated pulmonary arterial pressure in the majority, especially following exercise. This fact prompted the study of the surgically resected tuberculous lung with attention directed to the arterial system. Vascular lesions in pulmonary tuberculosis were observed in autopsy material by Brenner (1); the small muscular arteries near tuberculous foci were involved in a process of endarteritis obliterans and caseation; the changes were insufficiently widespread to cause vascular obstruction. Charr and Savacool (2) demonstrated, arteriographically and histologically, destruction of the vascular bed about tuberculous cavities. In several cases of progressive pulmonary tuberculosis, Terplan (3) found various specific old and recent endarteritic lesions, topographically related to the parenchymal lesions, in which the lumina were eccentrically narrowed. In two other cases he observed obliterating endarteritis of smaller arterial branches in areas of collapse-induration, with concentric obstruction of the lumen caused by the proliferation of the intima alone. He believed this represented a reactive change in the vessel wall conditioned by the collapse-induration and resulting in permanent local strain on the pulmonary circulation. Pagel and his associates (4) stated that the vascular lesions in tuberculosis consisted of nodules of epithelioid and giant cells and that intimal scars devoid of characteristic features occurred. Only in the acute miliary disease were larger intimal foci of caseation and liquefaction present. In the present study an attempt was made to determine the nature and extent of the vascular alterations in the surgical specimen, both in tuberculous and in remote areas. Consideration was given to the possible relationship of these changes to pulmonary hypertension and to the effect of collapse therapy.

MATERIAL AND METHODS

Surgical specimens from 85 unselected patients were used. The entire lung had been resected in 39 cases, and lobectomies had been performed in 46 cases. Material from segmental resections was not included. Forty-four patients were women, and 41 were men. The ages ranged from nineteen to sixty years, with 20 in the third decade and 42 in the fourth decade. Specimens from patients who had experienced no prior collapse therapy totaled 12. Twenty-one patients had had successful pneumothorax, pneumonolysis, or phrenic crush procedures only, for varying lengths of time. Twenty-three patients had had thoracoplasty or extrapleural Lucite plombage for less than one year. Twenty-nine patients had had thoracoplasties from one to eleven years previously.

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In all cases the specimen was fixed in formalin. Blocks were taken for imbedding in paraffin and sections were obtained not only from the walls of cavities and caseous foci but from all representative areas, including the hilus of the lung, the large and small bronchi, and the peripheral portions. A special attempt was made to include material from areas which were not grossly involved by the tuberculous process. The sections were stained routinely by hematoxylin and eosin. Many also were stained by the elastic fiber-van Gieson method. A few were stained for fat with Scharlach-R.

OBSERVATIONS

The large arteries near the hilus of the lung exhibited eccentric or diffuse intimal proliferation of varied degree, from slight to severely obstructive (figure 1). The intimal tissue was composed of fibroblasts or fibrocytes in a loose webby arrangement, without elastic fibers, and it occasionally contained a mucinous ground substance. Most of these loose intimal fibers stained yellow with the van Gieson stain. Light to moderate focal infiltrations of lymphocytes and some polymorphonuclear leukocytes were present in the intimal connective tissue bordering the internal elastic membrane in some cases. In a few instances, the intima was densely collagenous and poorly nucleated. The earliest change appeared to be swelling or straightening of the internal elastica. The media was less often and less severely altered; sometimes it was thickened, compressed, or showed slight diffuse or patchy fibrosis, and the elastic fiber component was little disturbed. Lipoid infiltrations were rarely observed, and plaque-like accumulations of foam cells were not seen.

The vessels were usually collapsed and tortuous, as might be expected in the collapsed lung. These large arteries were often present in dense scar tissue near the walls of chronically inflamed bronchi or near tuberculous nodules, but they where seldom entrapped in the walls of cavities. The medium-sized arteries were similar both in the changes which they showed and in their location in chronically inflamed or healed scar tissue. The small arteries were often more extensively and severely diseased. A porous spongy granulation tissue, poor in fibers but rich in lymphocytes, occluded some of them and was often canalized by small endothelial channels (figure 2). The majority of the medium-sized and small arteries which were completely obliterated, however, were present in the walls of cavities. When so situated, the media and adventitia were scarred and distorted, blending inconspicuously with adjacent granulation and fibrous tissue. Similar advanced destruction of vessels was rare in other areas.

A not unusual finding elsewhere was the recanalization of a small artery, which was filled with much loose fibroblastic tissue, by large channels rimmed by bands of collagenic tissue, thus producing the picture of vessels within a vessel (figure 3). Laminated thrombi were not observed in this material even in the walls of cavities. In a single case a small artery contained a polypoid fibrin clot which was covered by endothelium and was attached to a thin intima at one point. The smallest arteries and large arterioles exhibited severe concentric productive intimal changes of a similar nature (figure 4). The intima, together with the entire diffusely fibrotic or atrophic wall, was more frequently infiltrated by lymphocytes and lesser numbers of polymorphs and eosinophils.

The bronchial arterioles were, as described by Terplan (3), possessed of even, severely proliferated cellular-fibrous intima and lumina constricted to a thread-

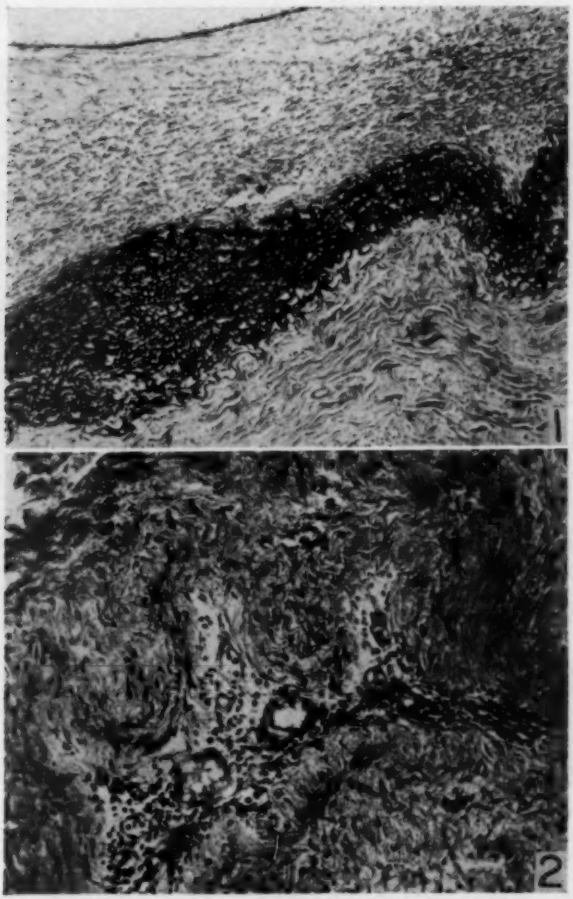

FIG. 1. Large hilar artery showing concentric fibrillar intimal proliferation, swollen media, and fibrotic adventitia. Elastic fiber-van Gieson stain. ×90.

FIG. 2. Obliterated and recanalized collapsed artery in collapsed, scarred parenchyma. Hematoxylin-eosin stain. ×175.

like caliber. Many of these had compressed media, but in others the entire vessel wall appeared much enlarged and densely collagenous (figures 5 and 6). The minute arterioles in collapsed or partially collapsed but otherwise normal

parenchyma showed less spectacular but definite changes, consisting most often of moderate fibrosis of the intima or broad enlargement of the adventitial fibrous incasement (figures 7 to 9). Such sclerotic channels were often patent or dilated. The vessels of precapillary size rather consistently showed extensive pale, uni-

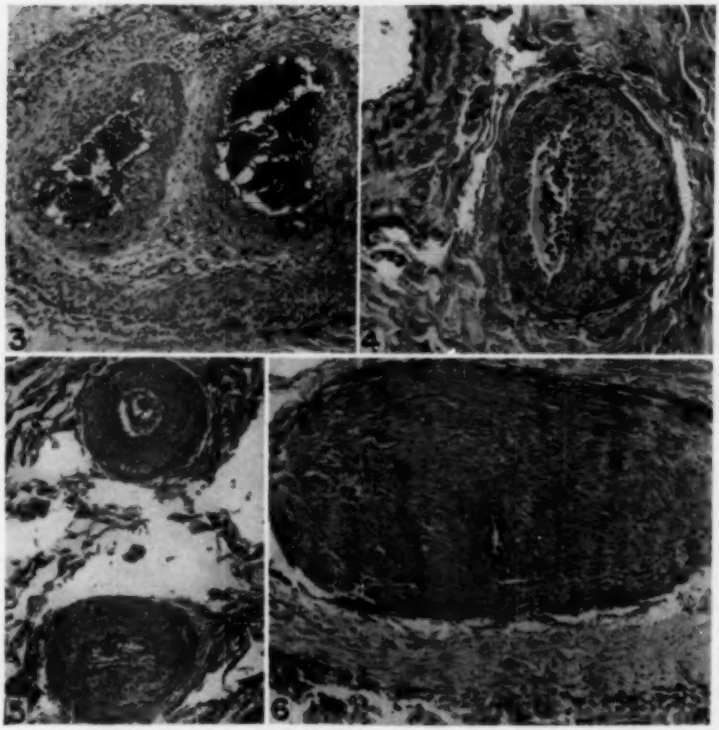

FIG. 3. Small obliterated artery showing "vessels within a vessel," present in a densely scarred area. Hematoxylin-eosin stain. ×90.

FIG. 4. Large arteriole with eccentric fibrocellular intimal growth in a large area of collapse and fibrosis. Hematoxylin-eosin stain. ×175.

FIG. 5. Peribronchial arterioles with concentric intimal proliferation. Hematoxylin-eosin stain. ×90.

FIG. 6. Arteriole near small bronchus possessing severely thickened collagenous wall and obstructed lumen. Hematoxylin-eosin stain. ×90.

form hyaline thickening of the wall. The arterial lesions in the different cases were remarkably consistent in quality but not in extent and degree, and changes characteristic of tuberculosis were rare. In only one instance was a multinucleated giant cell present in intimal granulation tissue. In another, a giant cell was imbedded in the adventitia of an artery situated in a fibrous scar.

It should be emphasized that the involved vessels were located not only in the walls of cavities and adjacent to caseous nodules but were present in other areas. In general, the surgical specimens showed very extensive pathologic alterations. They were often gnarled, carnified, or indurated, and seldom was

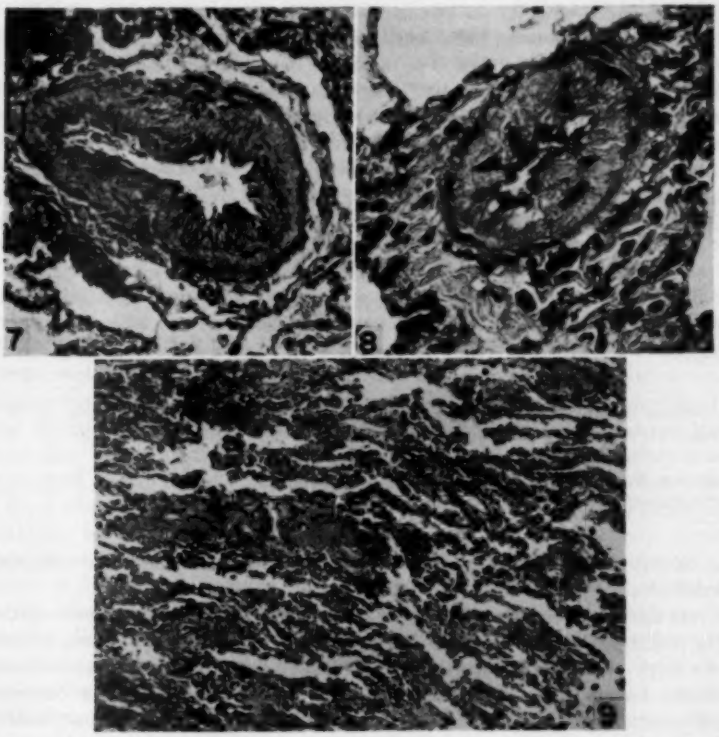

FIG. 7. Arteriole with fibrous intima in collapsed, normal parenchyma. Hematoxylin-eosin stain. ×175.

FIG. 8. Severely obstructed minute arteriole surrounded by collagen in otherwise normal parenchyma. Hematoxylin-eosin stain. ×450.

FIG. 9. Narrowed, diffusely fibrotic arterioles in collapsed lung tissue. Hematoxylin-eosin stain. ×90.

only an isolated cavity or localized fibrocaseous area present. Most specimens exhibited widespread fibrous scarring; interstitial fibrosis, with or without nonspecific chronic inflammation; widespread tuberculous bronchitis or histologically nonspecific bronchitis; peribronchial and endobronchial fibrosis in otherwise normal areas; moderate bronchiectasis; focal emphysema; and various combinations of these changes. The arterial lesions were frequent in all of these

diseased areas and were much less common in normal tissue. The minute arteriolar and precapillary changes, however, were often widespread in collapsed normal parenchyma in the periphery of the lungs.

No attempt was made to grade the severity or extensiveness of the vascular lesions rigidly, as such classification is subjective and the primary concern was to determine whether the changes were extensive or severe enough to be a factor in the production of pulmonary hypertension. The majority of specimens showed at least slight changes. The incidence of significant lesions is presented in table 1. The cases are grouped roughly according to the type of previous treatment. The term moderate is used when the endarteritic changes were unequivocal and when diseased vessels were observed in the majority of the sections. When approximately one-half or more of the vessels exhibited pronounced or obstructive changes, they were termed severe. The column of arterial changes refers to those vessels present in areas other than the walls of cavities.

TABLE 1

The Incidence of Significant Lesions

TYPE OF CASE	NUMBER OF CASES	ARTERIAL LESIONS		ARTERIOLAR LESIONS	
		Moderate	Severe	Moderate	Severe
No collapse therapy	12	4	0	3	0
Pneumothorax and/or phrenectomy	21	11	3	10	2
Thoracoplasty or extrapleural Lucite plombage, less than one year	23	8	7	8	2
Thoracoplasty, greater than one year	29	16	7	7	7

The column of arteriolar changes refers to those vessels in collapsed or relatively normal areas.

From the complete series of cases, 28 pneumonectomy specimens were selected, all of which possessed one relatively normal lobe, and the small vessels in these lobes were studied. Twenty-three exhibited widespread, at least moderate, sclerotic changes in the small arterioles and precapillaries. In 8 of these, however, significantly large microscopic areas of interstitial fibrosis or chronic pneumonitis were also interspersed through the normal parenchyma.

DISCUSSION

As is apparent, vascular changes were observed in the majority of specimens. The arterial changes were widespread, but were located mainly in scar tissue whether it was peribronchial, patchy in the parenchyma, or interstitial in location. It was our impression that the pathologic examination revealed much more extensive disease in most cases than was expected. To estimate quantitatively the percentage of the vessels involved, as well as the relative amount of diseased parenchyma, was impossible. One of the difficulties encountered was to obtain sections from normal areas, as the uninvolved collapsed parenchyma was disproportionately shrunken in relation to the diffuse or nodular fibrosis, the thick-

walled cavities, or patches of caseation. The majority of the sections showed some tuberculous or scarred foci widely distributed through the more grossly normal parenchymatous areas.

It appeared likely that the arteries were involved by direct encroachment of the tuberculous process on the vessel wall although classical lesions were rarely seen. The characteristic nonspecific lesions may well result from toxicity from tuberculous foci in the neighborhood, and caseation and tubercle formation probably occur only in an overwhelming infection. Moreover, identical vascular lesions were seen in the areas of active caseation, with the added elements of severe edema of the wall and more profound infiltration of the wall by leukocytes. The lesions were quite similar to those productive arterial changes observed in chronic tuberculous meningitis treated by streptomycin (5). It appeared likely, especially in the instances of chronic disease under treatment, that the widespread destructive fibrosis represented healed tuberculosis. The accompanying vascular sclerosis and endarteritis were therefore stigmatic of the previously active process. Pagel and associates (4) stated that tubercle bacilli reached the intima through the blood stream during bacteremia and produced nodular granulomatous intimal lesions and, if the local resistance was high, specific features were not seen. In our material the lesions were usually more diffuse, but the location of normal vessels adjacent to those showing acute panarteritis or sclerosis was not uncommon. Another factor to be considered is that chronic local tissue anoxia, which was probably present in many of these cases, may have stimulated parenchymal and arterial fibrosis. A factor in the production of the diffuse chronic nonspecific fibrosing interstitial inflammation so often observed may have been secondary infection. Geever, Neubuerger, and Rutledge (6) reported that vascular lesions occurred in acute and chronic atypical pulmonary inflammatory reactions. The nonspecific nature of the lesions in this series of cases is not inconsistent with the possibility of such infection. Viral infections may play a part; tissue anoxia is thought to favor viral infection. While the appearance of the large arteries was not like that of arteriosclerosis, the arterioles and precapillaries were similar to those observed in emphysema as well as to those in other organs in patients with long-standing systemic hypertension.

An interesting theoretical question is whether the vascular damage in the collapsed lung is of sufficient severity to affect pulmonary function on re-expansion. The incidence of widespread lesions was greater when collapse had been present for many years and greatest when thoracoplasty had been performed. In spite of extensive arterial changes and the constriction of minute arterioles by collagen cuffs, the lumen was usually patent and not infrequently was dilated. The functional result after re-expansion would appear to be limited mainly by the extent of the pleural, bronchial, and parenchymal change rather than by the presence of the vascular lesions.

Spain (7) showed that pulmonary hypertension with cor pulmonale was most frequently related to pulmonary fibrosis when the pattern was one of bronchiolar fibrosis with resultant obstructive emphysema. He also stated that involvement

of the pulmonary vascular bed will produce some degree of hypertension as well as impairment of nutrition of the lung. The fibrosis in tuberculosis may be of several patterns. Patients suffering from very extensive pulmonary tuberculosis exhibit relatively normal resting pulmonary artery pressure. In the normal person the pulmonary vascular bed is capable of distension to three times the normal caliber in order to accommodate the increased blood flow necessarily present in increased metabolic states. With a moderate degree of vascular sclerosis this compensatory mechanism is diminished, with resulting increased pulmonary artery pressure on exercise, very frequently to hypertensive levels. The results of cardiac catheterization studies performed on a group of these patients and reported elsewhere (8) showed that moderate increases in pressures from the normal were usual. No true correlation existed, however, between the degree of vascular alteration and the preoperative pulmonary artery pressures at rest or exercise. This may have been due to the fact that the amount of resected lung tissue did not represent by any means the total vascular bed involvement. The frequent presence of contralateral disease would indicate that vascular changes may also exist in unresected areas of the lungs in many of these cases. One frequently refers to such a situation as a predominantly unilateral process.

It has been said by Cournand and co-workers (9) that, following the resection of one lung, the remaining lung must assume the burden of the total pulmonary circulation in order to maintain adequate arterial oxygen saturation levels. At rest, such individuals with one lung maintain a pulmonary artery pressure within normal limits. On exercise of even slight to moderate degrees, the pressure usually rises to hypertensive levels. The postoperative cardiac catheterization findings in this series were bizarre, exhibiting no consistent pattern. From our present knowledge of the physiology of the pulmonary circulation, it can be concluded only that, while the pathologic changes in the vascular tree are of great importance, other factors which are incompletely understood at this time must play a significant role.

SUMMARY

In chronic pulmonary tuberculosis, productive endarterial lesions are common. The vascular lesions are present not only in the walls of cavities but are frequently widespread in areas of scar tissue, interstitial fibrosis, and bronchial and peribronchial fibrosis. The lesions are not specific but probably result mainly from acute and healing parenchymal tuberculosis although nonspecific inflammation may play a role. The arteriolar changes are frequently extensive in relatively normal or collapsed lung tissue and resemble those present in emphysematous lungs and in other organs in association with systemic hypertension. The incidence is greater in lungs previously treated by collapse and particularly in those collapsed by long-standing thoracoplasty. The extent of the vascular alterations necessitates their consideration as a factor in pulmonary hypertension. In the present series of cases, whenever the pulmonary arterial pressure was measured it was usually moderately elevated, but no correlation

was apparent between the degree of vascular change and the pulmonary arterial pressure.

SUMARIO

Estudio Histológico de los Vasos Sanguíneos en los Pulmones Tuberculosos Resecados Quirúrgicamente

En la tuberculosis pulmonar crónica, son comunes las lesiones endarteriales histógenas. Hay lesiones vasculares presentes no sólo en las paredes de las cavernas sino también frecuentemente esparcidas por las zonas de tejido cicatricial, fibrosis intersticial y fibrosis bronquial y peribronquial. Esas lesiones no son específicas, pero con toda probabilidad provienen de una tuberculosis parenquimática aguda y en vías de curación, aunque la inflamación anespecífica puede desempeñar un papel en ellas. Las alteraciones arteriolares son frecuentemente extensas en el tejido pulmonar relativamente normal o aplastado, semejando las presentes en los pulmones enfisematosos y en otros órganos en asociación con hipertensión general. La incidencia es mayor en los pulmones previamente tratados por el colapso y en particular en los aplastados por una toracoplastia de largo tiempo. La extensión de las alteraciones vasculares obliga a considerarlas como factor en la hipertensión pulmonar. En la actual serie de estudios, siempre que se midió la tensión arterial pulmonar, mostró por lo general moderada elevación, pero no había correlación aparente entre la intensidad de la alteración vascular y dicha tensión arterial.

RESUME

Etude histologique des vaisseaux sanguins dans le poumon tuberculeux, après résection chirurgicale

Les lésions endartérielles cicatricielles dans la tuberculose pulmonaire chronique sont fréquentes. Les lésions vasculaires sont présentes non seulement dans les parois des cavernes, mais elles s'étendent aussi fréquemment aux zones de tissus cicatriciels, fibrose interstitielle et fibrose bronchique et péribronchique. Les lésions ne sont pas spécifiques mais il est probable qu'elles représentent surtout le résultat d'une tuberculose aiguë du parenchyme en voie de cicatrisation, bien qu'un processus inflammatoire non spécifique puisse jouer un rôle. Les altérations des artérioles s'étendent souvent aux tissus du poumon relativement normaux ou collabés; elles ressemblent à celles du poumons emphysémateux ou á celles observées dans d'autres organes, en association avec l'hypertension artérielle. La fréquence la plus grande s'observe au niveau des poumons antérieurement traités par le collapsus et en particulier dans ceux collabés par une thoracoplastie de longue durée. L'étendue des altérations vasculaires demande qu'elles soient considérées comme un facteur d'hypertension pulmonaire. Dans l'étude actuelle, lorsque la tension artérielle pulmonaire a été mesurée, elle était habituellement modérément élevée, mais aucune corrélation n'est apparue entre le degré d'altération vasculaire et la pression artérielle pulmonaire.

REFERENCES

(1) Brenner, O.: Pathology of the vessels of the pulmonary circulation, Arch. Int. Med., 1935, *56*, 1189.

(2) Charr, R., and Savacool, J. W.: Changes in the walls of tuberculous pulmonary cavities, Arch. Path., 1940, *30*, 1159.

(3) Terplan, K.: Anatomical studies on human tuberculosis, Am. Rev. Tuberc., 1945, *51*, 321.

(4) Kayne, G. G., Pagel, W., and O'Shaughnessy, L.: Pulmonary Tuberculosis, Oxford Medical Publications, 1948.

(5) Ogura, G. I., and Denst, J.: The effect of streptomycin on the lesions of tuberculous meningitis, Dis. of Chest, 1950, *18*, 291.

(6) Geever, E. F., Neubuerger, K. T., and Rutledge, E. K.: Atypical pulmonary inflammatory reactions, Dis. of Chest, 1951, *19*, 325.

(7) Spain, D. M.: Patterns of pulmonary fibrosis as related to pulmonary function, Ann. Int. Med., 1950, *33*, 1150.

(8) Hurst, A., Dressler, S. H., and Denst, J.: The relationship between pathologic changes in blood vessels in resected lobes and lungs as correlated with pulmonary artery pressure changes recorded during cardiac catheterization, Dis. of Chest (in press).

(9) Cournand, A., Riley, R. L., Himmelstein, A., and Austrian, R.: Pulmonary circulation and alveolar ventilation-perfusion relationships after pneumonectomy, J. Thoracic Surg., 1950, *19*, 80.

THE VALUE OF FOLLOW-UP STUDIES OF CHILDREN WITH PRIMARY TUBERCULOSIS[1,2,3]

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(Received for publication May 16, 1951)

Follow-up studies of children with first-infection tuberculosis have taught us a great deal about the course and prognosis of the early phases of tuberculosis in the child. Reports from various centers emphasize the fact that the fundamental pattern of tuberculosis is the same everywhere in the world even though there may be minor variations in clinical pictures. Reports from different countries and even different parts of the same country, however, do not agree on the prognosis of primary tuberculosis in children. It seems probable that the severity of the disease in any given area reflects the general tuberculosis situation of the community and is related to the social and economic level of the majority of the tuberculous patients.

Myers was one of the first to point out the low incidence of serious complications of primary tuberculosis in children, but his statistics were collected in Minneapolis where the morbidity and mortality rate from tuberculosis in adults is outstandingly low. During the same period of time the tuberculosis rate in New York City was much higher than it was in Minneapolis; the tuberculous children in our prognosis study came from the congested homes of families of low economic levels. Even though 85 per cent of our tuberculous children were initially discovered by the same case-finding methods as were employed in Minneapolis, i.e., examination of contact cases and routine tuberculin testing, the mortality rate in our group from 1930 to 1940 was 23.9 per cent (1), whereas the rate reported by Tortone and others on Myers' group in Lymanhurst was 5.8 per cent (2).

The actual difference in rates is not important. What is important is the recognition of the fact that primary tuberculosis in children is always potentially a serious disease. Most children have light cases of first-infection tuberculosis without clinical evidence of illness. That fact does not change the potential import of the disease to other children, especially to those with more extensive evidence of primary tuberculosis.

NATURAL COURSE OF PRIMARY TUBERCULOSIS IN CHILDREN

Before the days of chemotherapy, many clinicians believed that a knowledge of the natural course and prognosis of primary tuberculosis in children was of

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interest but not of great practical importance. With the possibility of cure of serious complications, it becomes our duty to be aware of the clinical course of primary tuberculosis, and especially of the usual timing of complications, in order to get the best results from early diagnosis and early treatment of potentially fatal complications.

In the chemotherapy program at Bellevue Hospital, information gained from previous follow-up studies has aided the selection of cases for treatment and the evaluation of the results of specific therapy. Between 1930 and 1940, 622 consecutive cases of primary pulmonary tuberculosis were studied in the Children's Chest Clinic, and the survivors of this group continue under observation. One hundred and forty-nine, or 23.9 per cent, have died as a direct result of the first infection with tuberculosis. Meningitis caused 60 per cent of the deaths with less than 10 per cent additional mortality from miliary tuberculosis without meningitis. One-quarter of the deaths were due to protracted hematogenous tuberculosis and to locally progressive primary tuberculosis in approximately equal proportions. These four complications of primary tuberculosis thus accounted for 95 per cent of the mortality. When streptomycin first became available the decision was made, therefore, to use it in primary tuberculosis only when one of the four major complications was present. In the two years prior to streptomycin, the case fatality rate from primary tuberculosis was 18 per cent. During the past four years, the case fatality rate has been less than 3 per cent although less than 25 per cent of the ward population received streptomycin.

FACTORS WHICH INFLUENCE PROGNOSIS

Further study of the 622 cases of primary pulmonary tuberculosis revealed that three factors influence the prognosis of an individual case: the age of the child when he developed his first infection with tuberculosis, the duration of the primary tuberculosis, and, to a lesser degree, the extent of the tuberculous process.

Age of patient: The age of the patient is always important in an estimation of prognosis. More than half of the infants first diagnosed when less than six months of age died of tuberculosis. Of the infants who were between one and two years of age when tuberculosis was diagnosed, only 28 per cent died of the disease; the mortality rate of children five through nine years of age was 14 per cent (figure 1).

Age of lesion: The age of the tuberculous lesion is also an important factor in prognosis. The interval between the diagnosis of tuberculosis and death in our prognosis study is shown in figure 2. Approximately 90 per cent of the deaths occurred within a year of the first diagnosis of primary tuberculosis, three-quarters within six months, and almost 60 per cent within three months. Therefore, a child who has survived a year after a diagnosis of primary tuberculosis is unlikely to die from complications of the disease; conversely, the inference is obvious that one should keep a child with a known recent onset of primary tuberculosis under close supervision so that symptoms suspicious of a serious complication may be noted promptly.

Extent of lesion: The influence of the size of the primary complex on prognosis is more difficult to determine; the differential diagnosis between a shadow cast on a roentgenogram by the parenchymal component of the primary complex and that of an obstructive lesion secondary to tuberculous nodes is not always an easy one. In our series, however, an attempt was made to measure the size

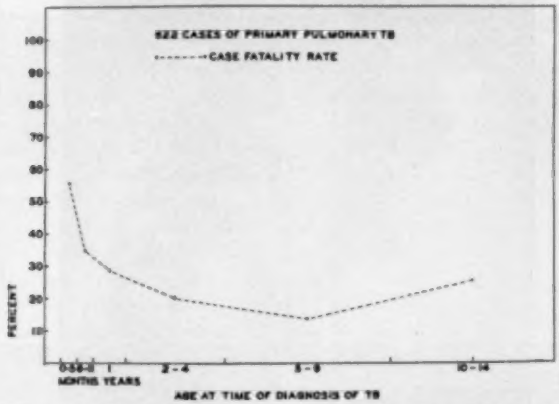

FIG. 1. Case fatality rate of primary pulmonary tuberculosis by age.

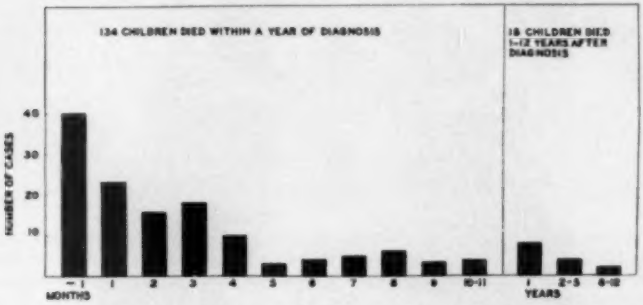

FIG. 2. Interval between diagnosis of tuberculosis and death.

of the primary complex on the roentgenogram and to relate the results to prognosis. The mortality rate in the children with the largest lesions was more than double the rate in children with small primary foci. Fortunately, only 8 per cent of the entire group had very large primary infections.

FOLLOW-UP STUDIES

Follow-up studies may also be of value in shedding light on the endogenous or exogenous origin of "reinfection" tuberculosis. For this purpose, 1,000 con-

secutive cases of primary pulmonary tuberculosis have been followed in the Children's Chest Clinic of Bellevue Hospital. This group includes the 622 children used for the study of immediate prognosis and 378 additional children with roentgenographic evidence of primary tuberculosis which had already begun to

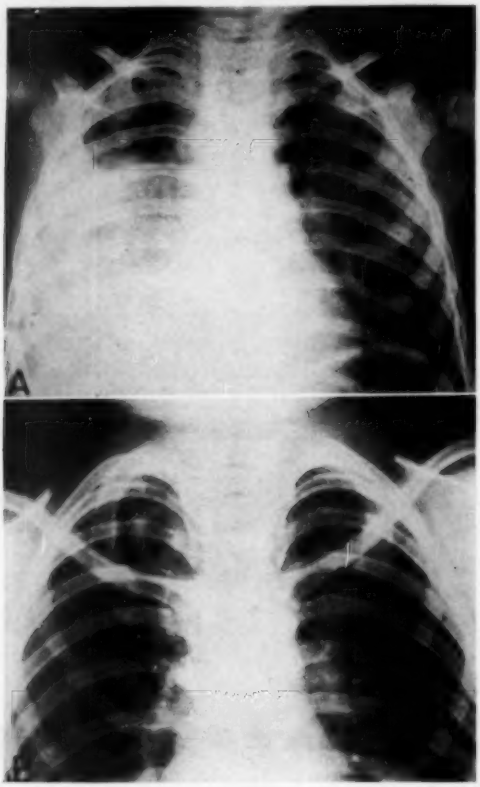

FIG. 3. Patient A. B. (A) Primary pulmonary tuberculosis in right lung in infancy. Patient not followed. (B) Reinfection type of tuberculosis with cavitation and sputum positive for *M. tuberculosis*. Patient is eight years old. Roentgenogram was taken because of recurrent fever.

calcify or was well calcified when first observed. Patients are followed until the age of 25 years and more than 90 per cent of the survivors of this entire group have remained under observation to date. Follow-up observations of this group have shown the development of chronic pulmonary tuberculosis in 8 per cent of the survivors at intervals of one to fourteen years after the first infection. More

than half of the cases occurred in adolescents; more than twice as many in females as in males; in most girls, the first roentgenographic abnormality was found within two years of menarche.

The results of follow-up studies on large groups of cases have demonstrated the value of follow-up of each individual child with primary tuberculosis. It is

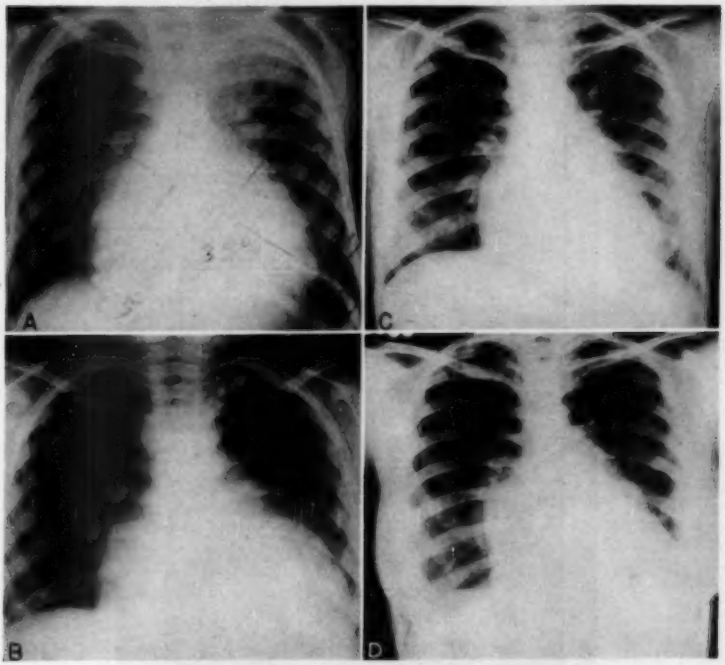

FIG. 4. Patient D. B. (A) Primary pulmonary tuberculosis of left upper lobe in a child five years old with rheumatic heart disease. Roentgenogram obtained in 1932. (B) Irregular densities left apex, probably residual caseation and calcification. Roentgenogram obtained in 1935. (C) Marked diminution in size and extent of densities at left apex. Roentgenogram obtained in 1937. (D) Essentially clear left apex. Roentgenogram obtained in 1941 when patient was fourteen years old.

important to follow a child with a recent primary tuberculosis closely in order to watch for complications during the first year after infection has taken place. Even during the so-called "safe period," one year after infection to adolescence, however, it is desirable to examine and obtain roentgenograms of the child at intervals because of the individual variation in time of development of late complications. It is particularly important to follow children regularly during the period of greatest incidence of chronic pulmonary tuberculosis, but the possi-

bility that an individual patient may develop evidence of chronic pulmonary tuberculosis long before adolescence must not be forgotten (figure 3).

Follow-up observations may also be especially important for the patient who shows a variation from the usual method of healing of primary pulmonary tuberculosis. Serial roentgenographic studies of the survivors of the 622 cases usually

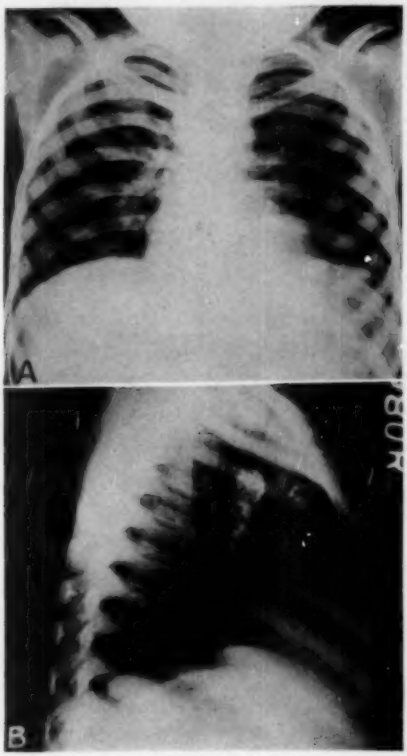

FIG. 5. Patient D. P. (A) Roentgenogram of a child eight years old with minimal calcification right root. (B) Lateral view suggested extensive nature of original infection and directed attention to diagnosis of tuberculous enteritis, later proved by autopsy.

showed evidence of calcification but, occasionally, large parenchymal and lymphatic lesions healed without evidence of calcification. In such patients, follow-up is particularly important since the tuberculous nature of late complications might not be suspected and diagnosis might be unduly delayed. When the past history of tuberculosis is unknown to the physician, a late diagnosis is often made of tuberculous meningitis; enlarged nodes are not infrequently mistaken for

Hodgkin's disease or lymphosarcoma. In most cases once calcification had reached its maximum, it thereafter remained unchanged during the period of observation. In a few children, marked diminution of shadows apparently due

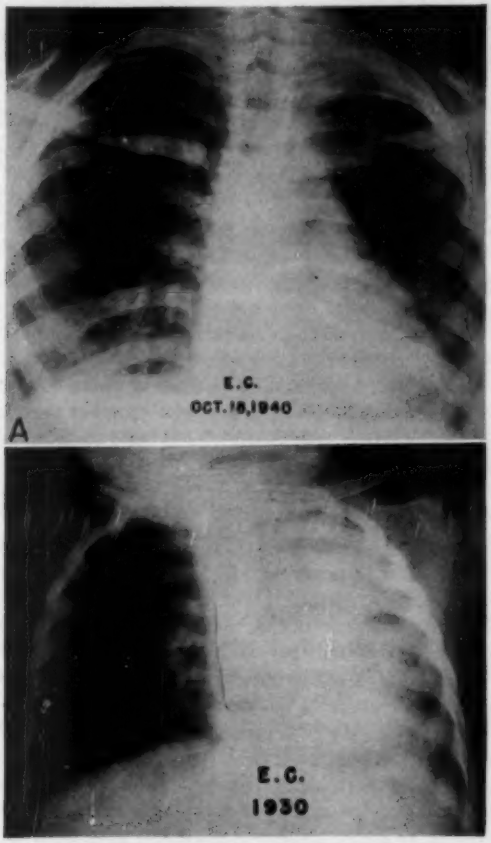

FIG. 6. Patient E. C. (A) Roentgenogram of twelve-year-old girl with clouding of inner half of left apex. Admitted as chronic pulmonary tuberculosis because of roentgenogram, constant fine rales at apex, and positive Mantoux. (B) Roentgenogram of same patient at two years of age with evidence of obstruction of left upper lobe, suggesting that (A) represented fibrotic residual of original lesion, substantiated by clinical course and negative laboratory data.

to calcification was observed in roentgenograms obtained as part of the follow-up examination (figure 4). When evidence of calcification is seen only in lateral or other views of the lungs, failure to follow a case of primary tuberculosis may lead

to failure to diagnose subsequent tuberculosis (figure 5). Some cases of primary tuberculosis heal without roentgenographic evidence of calcification but with fibrosis and streaking. In such instances, unless one has followed the child from the time of his primary infection, a mistaken diagnosis of reinfection tuberculosis may be made. This mistake is particularly apt to occur when the primary tuberculosis was in an upper lobe or associated with an obstructive lesion (figure 6).

It is always desirable to know when a tuberculous infection has taken place in a child but, unless the child is a known contact or is admitted to a hospital where tuberculin tests are done as part of a general examination, an early diagnosis is often not made. It might be well for those interested in tuberculosis control to stimulate pediatricians who follow children from birth through adolescence to do routine tuberculin tests as part of regular examinations and to examine roentgenographically all positive reactors at regular intervals. Since the cost of finding cases of pulmonary tuberculosis by surveys of children less than 15 years of age is usually considered prohibitive by public health authorities, this might be a means of early diagnosis of cases not found by contact examination. This type of follow-up observation of the well child as a means of early diagnosis of tuberculosis might help to prevent the tragedy of finding advanced tuberculosis on a first examination. The total number of cases of pulmonary tuberculosis in the pre-adolescent years may be few, but each case found at this age is important because of the high mortality rate in the untreated cases and the risk of contagion for other school children.

SUMMARY

Long-term follow-up observation of children with primary tuberculosis has many uses. It is of value in studying the course and immediate prognosis of primary tuberculosis and serves as a useful background for selection of cases for chemotherapy and for evaluation of the results of treatment by comparison with untreated cases observed over long periods of time. Long-term follow-up observation is also of great value in obtaining evidence regarding the pathogenesis of chronic pulmonary tuberculosis in adolescence and early adult life. Continued observation is important to the best care of each individual child who has evidence of primary tuberculosis. It ensures early recognition of signs and symptoms of important complications. Routine roentgenograms, especially during adolescence, should lead to early diagnosis of chronic pulmonary tuberculosis if the patient falls in the small group which is destined to have the reinfection type of disease. Finally, a knowledge of the patient's history may prevent mistakes in diagnosis due to lack of knowledge of the course of his disease.

SUMARIO

El Valor de los Estudios de Observación Subsiguiente en los Niños con Tuberculosis Primaria

La observación subsiguiente a largo plazo de los niños con tuberculosis primaria sirve muchos propósitos. Resulta útil para estudiar la evolución y el

pronóstico inmediato de dicho estado y ofrece una base práctica para la selección de casos para quimioterapia y para la justipreciación de los resultados terapéuticos mediante la comparación con casos no tratados mantenidos en observación durante períodos prolongados de tiempo. También resulta muy útil dicha observación para acopiar datos relativos a la patogenia de la tuberculosis pulmonar crónica en la adolescencia y en la vida adulta temprana. La observación continua es importante para la mejor asistencia de cada niño dado que muestra signos de tuberculosis primaria. Asegura el reconocimiento temprano de los signos y síntomas de complicaciones importantes; las radiografías sistemáticas, sobre todo durante la adolescencia, deben conducir al diagnóstico temprano de la tuberculosis pulmonar crónica si el enfermo corresponde al pequeño grupo destinado a tener la forma de reinfección de la dolencia; y por fin, el conocimiento de la historia del sujeto puede impedir errores de diagnóstico, debidos al desconocimiento de la evolución de la enfermedad en el mismo.

RESUME

La valeur de la mise en observation des enfants atteints de tuberculose primaire

L'exercice prolongé de la surveillance médicale chez l'enfant atteint de tuberculose primaire a de multiples avantages. Cette mise en observation permet d'étudier l'évolution et le pronostic immédiat de la tuberculose primaire; elle sert de base à la sélection des cas réservés à la chimiothérapie et à l'évaluation des résultats de la thérapeutique, par rapport aux cas non traités soumis à une mise en observation prolongée. La longue durée de la mise en observation a également une grande valeur pour fournir des données sur la pathogénèse de la tuberculose pulmonaire chronique dans l'adolescence et au début de l'age adulte. L'observation continue et les examens répétés sont importants pour le bien de chaque enfant présentant une atteinte de tuberculose primaire. Les manifestations pathologiques et les symptômes des complications importantes sont ainsi précocement reconnus; les radiographies, surtout dans l'adolescence, favorisent le diagnostic précoce de la tuberculose pulmonaire chronique lorsque le malade appartient à la catégorie du petit groupe qui est destiné à présenter la forme de réinfection de l'affection; enfin, une connaissance parfaite de l'histoire du malade peut prévenir les erreurs de diagnostic résultant de l'absence d'informations sur le cours de la maladie.

REFERENCES

(1) Lincoln, E. M.: Course and prognosis of tuberculosis in children, Am. J. Med., 1950, *9*, 623.

(2) Tobtone, J., Chattas, A., Myers, J. A., Stewart, C. A., and Streukens, T.: Tuberculosis in children less than 6 years of age, Am. J. Dis. Child., 1939, *58*, 92.

THE EFFECT OF ADRENOCORTICOTROPIC HORMONE (ACTH) ON PNEUMONIA INDUCED WITH TUBERCULIN IN THE LUNGS OF SENSITIZED RABBITS[1,2,3]

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INTRODUCTION

Adrenocorticotropic hormone (ACTH) and cortisone reduce or abolish the reaction to the tuberculin skin test in man (1, 2) and animals (3, 4, 5). Primary tuberculous infections in rats (6), guinea pigs (7), and rabbits (5) have been treated with cortisone. The tuberculous lesions in rats and guinea pigs were more extensive in the treated animals than in the controls, while the lesions in cortisone-treated rabbits were more numerous but of smaller size. A few patients with pulmonary and laryngeal tuberculosis have been treated with either ACTH or cortisone. In these patients the initial improvement was dramatic, but a relapse followed withdrawal of the drug (2). This relapse might have resulted from the return of tuberculin sensitivity, the continuous growth of tubercle bacilli, or some alteration in tissue resistance of the host induced by the hormone therapy. Obviously it would be valuable to study each of these factors separately.

In the present study an investigation was made of the effect of ACTH treatment on tuberculin-induced pneumonitis in sensitized rabbits.

MATERIALS AND METHODS

Forty rabbits, including animals of both sexes weighing between 2,000 and 3,000 gm., were sensitized to tuberculin by the subcutaneous injection of 1 ml. of a homogeneous suspension of living, avirulent tubercle bacilli (*Mycobacterium tuberculosis* R1v, Saranac strain). Thirty to forty days were allowed for sensitivity to develop after which the rabbits were used in four separate experiments.

Experiment I: Ten sensitized rabbits were etherized, the trachea of each exposed, and 0.25 ml. (250 mg.) of undiluted Old Tuberculin Koch (Lederle) was injected with a tuberculin syringe and a 24-gauge hypodermic needle. Each rabbit was held on its right side with the head raised to allow the tuberculin to flow into the right upper lobe. Five of the animals received ACTH (Armour)[4] in divided doses every six hours, totaling 10 mg. of drug each twenty-four-hour period. The first dose was given six hours prior to injection of OT.

At twenty-four-hour intervals, following the intratracheal injection of OT, a pair of rabbits, one treated and one control, was sacrificed by intravenous injection of 180 mg. of pentobarbital sodium. The lungs were dissected free, photographed in kodachrome and

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[1] From the Department of Bacteriology, Duke University School of Medicine, Durham, North Carolina.

[2] Presented before the Medical Session, as part of the *Symposium on Cortisone and ACTH*, at the 47th annual meeting of the National Tuberculosis Association, Cincinnati, Ohio, May 15, 1951.

[3] Supported, in part, by a Frederick M. Hanes Research Fellowship grant.

[4] The ACTH used in these experiments was furnished through the courtesy of Mr. Irby Bunding, Armour and Company, Chicago, Illinois.

black and white, and fixed in Helly's solution. Sections were cut from areas showing greatest involvement and stained with hematoxylin and eosin.

Experiment II: Twelve sensitized rabbits were injected with 0.25 ml. of diluted OT (2 parts OT, 1 part isotonic saline, and 1 part glycerin to equal 125 mg. OT) and the animals held on their left sides with heads elevated to achieve localization in the left lower lobe. Six were treated with ACTH and the remainder retained as controls. Pairs of treated and untreated rabbits were sacrificed on the first, second, and fourth days; two pairs on the fifth day and the last pair on the seventh day. The ACTH treatment regimen was the same as in *Experiment I* for all test animals except that one of those killed on the fifth day received no ACTH the previous twenty-four hours, and the one killed on the seventh day received no ACTH the previous seventy-two hours. Photographs and sections were prepared as previously described.

Experiment III: Ten sensitized rabbits were treated exactly as in *Experiment I* except that OT diluted with isotonic saline to contain 100 mg. in 0.25 ml. was used. Pairs were sacrificed the first, fourth, sixth, thirteenth, and seventeenth days after intratracheal OT injections. The test animal of the pair killed the thirteenth day received no ACTH after the end of the ninth day, and the test animal killed the seventeenth day received no ACTH after the end of the thirteenth day. Dosage schedule and methods of photographing and sectioning were the same as in *Experiment I*.

Experiment IV: Eight sensitized rabbits were treated as in *Experiment I*. Pairs of treated and untreated animals were sacrificed the third, fifth, seventh, and ninth days after injection. No test animal received ACTH after the third day. Diagrams were made instead of photographs, but all other methods of study were the same as in previous experiments.

Controls: Four normal, nonsensitized rabbits were injected intratracheally with 0.25 ml. (250 mg.) of undiluted OT; 2 were sacrificed at twenty-four hours; and 2 at forty-eight hours. Photographs and sections were made as previously described.

RESULTS

Experiment I: On the first day following the introduction of tuberculin into the lungs of sensitized rabbits, the control (figure 1, left) showed a gross early red pneumonic consolidation of the right upper lobe, while the ACTH animal (figure 1, right) had only spots of slight brownish discoloration in the right upper lobe. Microscopically (figures 4 and 5), the quality of reaction was the same in both animals, consisting of intra-alveolar hemorrhage, highly cellular inflammatory exudate, and thickening of the alveolar septae. On subsequent days the reaction became more severe and, although it was always visible grossly in the ACTH animals, it was estimated to be only 10 to 40 per cent as extensive as in the controls. The most severe reaction in the controls was seen on the fourth day when the control animal (figure 2, left) showed a 1 cm. necrotic area surrounded by a 1 cm. ring of dense purple consolidation. The ACTH animal on this day (figure 2, right) showed a markedly smaller gross reaction. Microscopically, the control (figure 6) showed a sharply demarcated area of complete necrosis with an underlying area of consolidation with complete obliteration of normal lung architecture and a conglomeration of polymorphonuclear leukocytes and mononuclear cells. While the test animal (figure 7) showed little reaction at the pleural surface, there was a deeper area of extensive consolidation qualitatively similar to that surrounding the necrotic area in the control. By the fifth day the last pair showed minimal lesions, those in the ACTH animal consisting only of streaks across the right upper lobe.

Experiment II: In *Experiment II*, post-mortem examination of pairs of rabbits sacrificed on the first, second, and fourth days confirmed the findings in the first experiment. On the fifth day two pairs were killed. One of the test animals had received no ACTH for the previous twenty-four hours; the other received ACTH every six hours until time of sacrifice. There were no demonstrable differences in the extent or degree of reaction in these two animals although both showed far less reaction than the two controls. The last pair was killed on the seventh day after injection of OT, the test animal being deprived of ACTH therapy for three days prior to death. The control animal on the seventh day showed no gross evidence of reaction (figure 3, left), the lesion having completely cleared, while the test animal (figure 3, right) had developed a fresh red consolidation simulating grossly the first day's reaction in a control animal (figure 1, left). Microscopically, the control (figure 9) showed resolution of the pneumonic reaction with a moderate degree of thickening of alveolar septae still present and a moderate number of polymorphonuclear leukocytes and a greater number of mononuclear cells within the alveoli. The test animal (figure 10) did not have the extensive involvement suggested by the gross lesion, but there were an increased intra-alveolar cellular exudate and hemorrhage, marked thickening of alveolar septae, and, in areas of less extensive involvement, evidence of the resolution of the pre-existent reaction.

Experiment III: Examination of pairs of rabbits sacrificed on the first, fourth, and sixth days after injection of OT confirmed the results noted in *Experiments I* and *II*. The remaining two pairs were killed on the thirteenth and seventeenth days after intratracheal OT. The test animal of each pair which had received no ACTH for three days prior to death again showed fresh new areas of consolidation while the controls showed grossly no remaining evidence of reaction. In neither case was the new consolidation as extensive as the initial reaction in sensitized controls but was as great as the average reaction obtained in ACTH animals under active treatment during the first five days of the reaction, e.g., figure 2, right.

Experiment IV: In this experiment, 4 sensitized rabbits received ACTH for three days and pairs of treated and untreated animals were sacrificed the third, fifth, seventh, and ninth days after injection of OT. When ACTH was withdrawn as early as the third day of the experiment, somewhat less protection was af-

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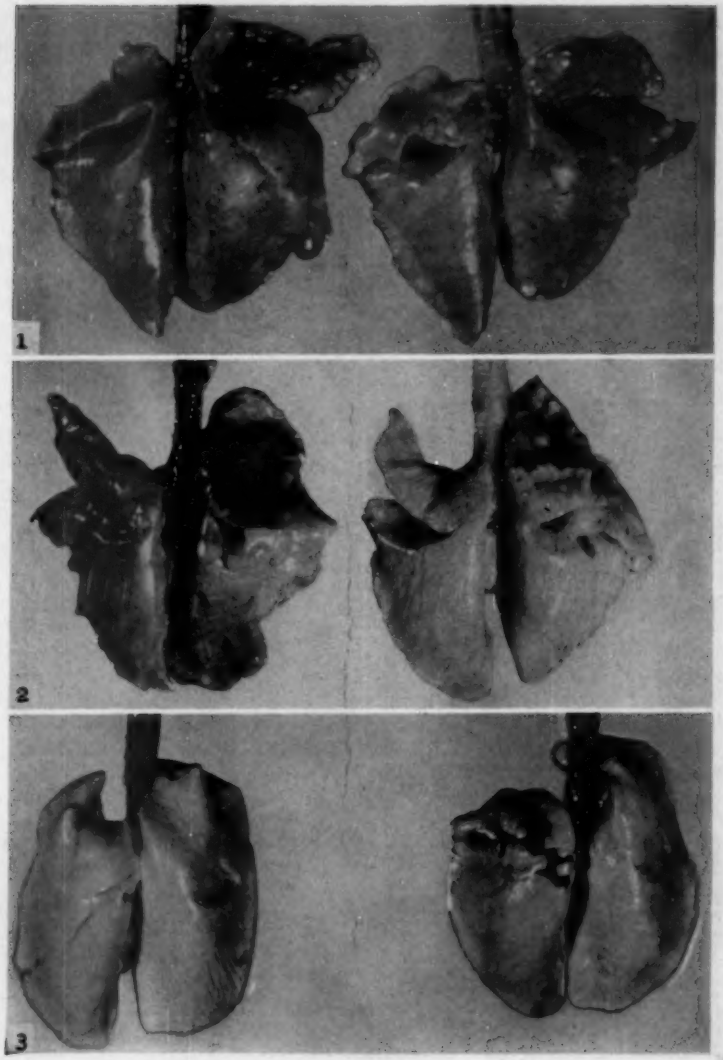
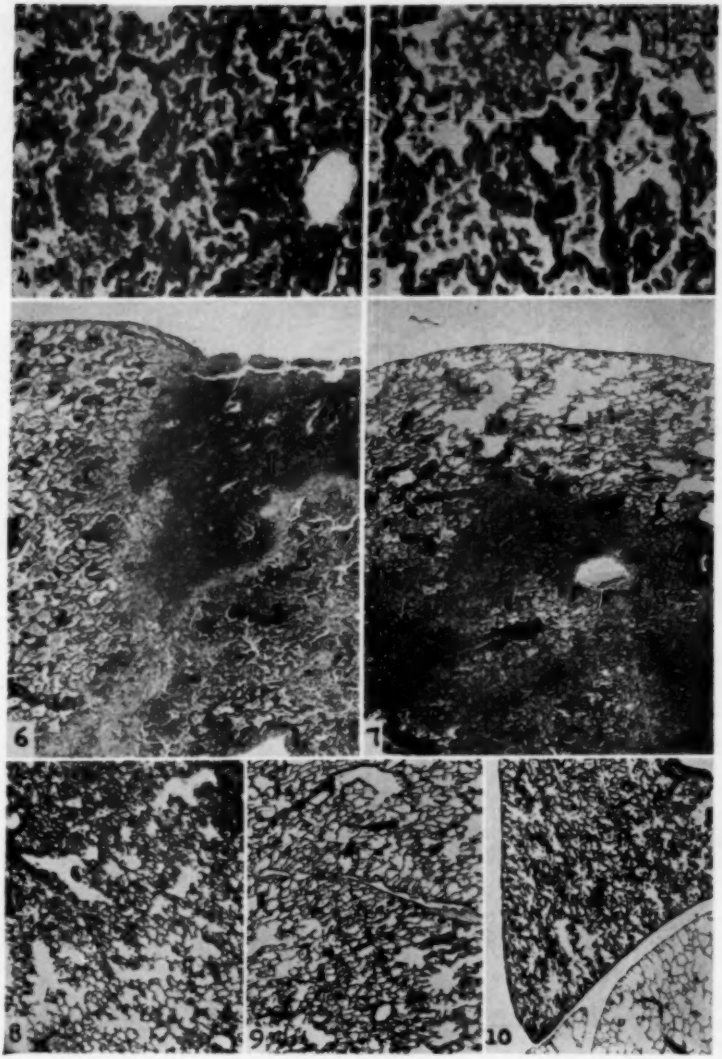
FIG. 1. (Left) Lungs from sensitized control killed 24 hours after intratracheal OT showing extensive fresh red consolidation. (×1). (Right) Lungs from sensitized test animal 24 hours after OT, protected from a major reaction by ACTH. (×1)

FIG. 2. (Left) Lungs from sensitized control fourth day after OT with a large area of necrosis in the center of hemorrhagic consolidation. (×1). (Right) Lungs from sensitized test animal showing less consolidation and no necrosis. (×1)

FIG. 3. (Left) Lungs from sensitized control on the seventh day after OT showing complete clearing of the reaction without treatment. (×1). (Right) Lungs from sensitized test animal on the seventh day after OT and the third day after cessation of ACTH treatment showing a fresh new hemorrhagic consolidation. (×1)



FIGS. 1-3



FIGS. 4–10

forded the treated animals, but there was also less recurrence of reaction after cessation of treatment.

Controls: The 2 normal nonsensitized rabbits which were sacrificed twenty-four hours after receiving 250 mg. of OT intratracheally had a few small scattered areas of erythema which on microscopic examination revealed some alveolar septal thickening and minimal alveolar exudate (figure 8). The pair killed after forty-eight hours showed no gross evidence of reaction.

COMMENT AND SUMMARY

Forty rabbits were sensitized by a subcutaneous injection of *Mycobacterium tuberculosis* R1v. Old Tuberculin in doses of 10 to 250 mg. was introduced into the trachea and allowed to flow into the lungs by gravity. One-half of the animals were treated with ACTH and the remainder served as controls. The rabbits were killed in pairs of treated and untreated animals one to seventeen days after the introduction of tuberculin. Four normal, nonsensitized rabbits received 250 mg. OT intratracheally and were killed at twenty-four and forty-eight hours.

The lungs of the nonsensitized rabbits showed a few small areas of erythema at twenty-four hours after injection but no gross evidence of reaction after forty-eight hours.

The sensitized rabbits which did not receive ACTH therapy showed definite red consolidated areas twenty-four hours after the introduction of tuberculin into the lungs. The maximum reaction, which included necrosis, was reached about the fourth day of the experiment. All gross evidence of pulmonary infiltration disappeared after six to twelve days.

The sensitized rabbits which were treated with ACTH showed consistently less extensive reaction than the untreated controls. It was estimated that the hormone had reduced the amount of reaction 60 to 90 per cent.

FIG. 4. Photomicrograph of a section from the lung shown in figure 1, left. Thickening of alveolar septae and a cellular hemorrhagic exudate with early consolidation are prominent. (×600)

FIG. 5. Photomicrograph of a section from the lung shown in figure 1, right. This section was taken from the area of most intense involvement and shows the same changes as figure 4. (×600)

FIG. 6. Photomicrograph of a section from the lung shown in figure 2, left, taken at the edge of the necrotic area to show minimally affected tissue and pleura at the left, a sharply demarcated area of necrosis at the right, and the underlying dense cellular consolidation at the lower right (cf. area of greatest involvement, figure 7). (×60)

FIG. 7. Photomicrograph of a section from the lung shown in figure 2, right, with consolidation resulting in loss of normal lung architecture, but with no necrosis. (×60)

FIG. 8. Photomicrograph of the area of greatest reaction in the lung of a nonsensitized control 24 hours after OT showing minimal cellular exudate and moderate alveolar septal thickening. (Gross specimen is not illustrated.) (×60)

FIG. 9. Photomicrograph of a section of the lung shown in figure 3, left, with resolution of consolidation. (×60)

FIG. 10. Photomicrograph of a section of the lung shown in figure 3, right, showing exacerbation of the reaction as compared with figure 9 and the unaffected lung tissue in the lower lobe subadjacent. (×60)

When ACTH was withdrawn between the fourth and the thirteenth days and the rabbits were sacrificed three days after cessation of treatment, a fresh acute pneumonic consolidation occurred which was similar in appearance but less extensive and severe than the initial reactions in the control rabbits.

Treatment with adrenocorticotropic hormone (ACTH) reduced significantly the amount of reaction induced by the introduction of Old Tuberculin into the lungs of sensitized rabbits.

When ACTH was withdrawn there was sometimes a recurrence of the pneumonic reaction in the lungs, but in no instance was the extent or severity of this reaction comparable to the initial lesions in the controls, suggesting that the tissues of the treated rabbits had not been altered sufficiently by the hormone therapy to give an exaggerated reaction to the residual amount of tuberculin remaining in the lungs.

SUMARIO

El Efecto de la Hormona Adrenocorticotropa (ACTH) sobre la Neumonía Inducida con Tuberculina en los Pulmones de Conejos Sensibilizados

El tratamiento con hormona adrenocorticotropa (ACTH) redujo significativamente la intensidad de la reacción evocada por la introducción de Tuberculina Antigua en los pulmones de conejos sensibilizados.

Al suspender la ACTH, hubo a veces recurrencia de la reacción neumónica en los pulmones, pero en ningún caso fueron la extensión o la gravedad de la reacción comparables a las lesiones iniciales en los testigos, indicando esto que los tejidos de los conejos tratados no habían sido alterados suficientemente por la hormonoterapia para acusar una reacción exagerada al residuo de tuberculina presente en los pulmones.

RESUME

Action de l'hormone corticotrope (ACTH) sur la pneumonie par inoculation de tuberculine dans le poumon du lapin sensibilisé

Le traitement par l'hormone corticotrope a réduit de façon appréciable l'importance de la réaction déterminée par l'introduction de la tuberculine de Koch (Old Tuberculin) dans le poumon du lapin sensibilisé.

Quand l'administration de l'ACTH a été supprimée, on a observé parfois une reprise de la réaction pulmonaire, mais dans aucun cas cette réaction n'a atteint degré de gravité comparable à celui des lésions initiales observées chez les animaux-témoins, ce qui permet de supposer que les tissus du lapin traité n'avaient pas été modifiés de facon suffisante par le traitement hormonal pour qu'une réaction excessive à la quantité résiduelle de la tuberculine demeurée dans le poumon se produise.

REFERENCES

(1) Long, J. B., and Favour, C. B.: The ability of ACTH and cortisone to alter delayed type bacterial hypersensitivity, Bull. Johns Hopkins Hosp., 1950, *87*, 186.

(2) (a) Tompsett, R., LeMaistre, C., Muschenheim, C., and McDermott, W.: Effects of ACTH on tuberculosis in humans, J. Clin. Investigation, 1950, *29*, 849.
(b) LeMaistre, C., Tompsett, R., Muschenheim, C., Moore, J. A., and McDermott, W.: Effects of adrenocorticotropic hormone and cortisone in patients with tuberculosis, J. Clin. Investigation, 1950, *30*, 445.
(3) Harris, S., and Harris, T. N.: Effect of cortisone on some reactions of hypersensitivity in laboratory animals, Proc. Soc. Exper. Biol. & Med., 1950, *74*, 186.
(4) Carlisle, J. M., Gibson, A., and Schmatolla, E.: Cortisone, Post.-Grad. M. J., 1950, *8*, 113.
(5) Lurie, M. B., Zappasodi, P., Dannenberg, A. M., Jr., and Swartz, I. B.: Constitutional factors in resistance to infection: The effect of cortisone on the pathogenesis of tuberculosis, Science, 1951, *113*, 234.
(6) Michael, M., Jr., Cummings, M. M., and Bloom, W. L.: Course of experimental tuberculosis in the albino rat as influenced by cortisone, Proc. Soc. Exper. Biol. & Med., 1950, *75*, 613.
(7) Spain, D. M., and Molomut, N.: Effects of cortisone on the development of tuberculous lesions in guinea pigs and on their modification by streptomycin therapy, Am. Rev. Tubere., 1950, *62*, 337.

ATTEMPTS AT PASSIVE TRANSFER OF TUBERCULIN TYPE OF SENSITIVITY WITH LIVING GRANULOCYTES[1,2]

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INTRODUCTION

Homologous passive transfer of the tuberculin type of sensitivity with living cells of tuberculin-sensitive guinea pigs and human beings has been accomplished in recent years by several groups of workers (1–7). In some of these experiments the cells of the tuberculin-sensitive donors were transferred by injecting them into the peritoneal cavities (1–5) or into the blood stream (1) of nontuberculin-sensitive recipient guinea pigs. In other instances (6, 7) the local passive transfer technique of Prausnitz and Küstner (8) was used.

The criterion chosen to indicate successfully accomplished transfer of the sensitivity was either cutaneous (1–3, 5–7) or systemic (4) response of the cell recipients to tuberculin.

The leukocyte suspensions used in the transfer of tuberculin sensitivity in man (7) were obtained from the blood of human cutaneous reactors to tuberculin by the use of the method of Minor and Burnett (9). This method allows the separation of leukocytes from erythrocytes. Since further separation of the various species of leukocytes was not carried out, it is likely that the cell suspensions used in these experiments contained a much higher percentage of granulocytes than those used by the other workers who prepared mononuclear cell suspensions from induced peritoneal exudates, lymph nodes, or splenic tissue of tuberculin-sensitive guinea pigs.

These observations as well as certain theoretical considerations suggest that the cells involved in the passive transfer of tuberculin-type sensitivity are of the mononuclear variety. There is, however, no published evidence of the role of granulocytes in this respect.

It seemed, therefore, desirable to carry out experiments to determine whether or not tuberculin-type sensitivity can be transferred with living granulocytes of tuberculin-sensitive guinea pigs.

MATERIALS AND METHODS

Male, albino guinea pigs weighing between 650 and 900 gm. were employed as cell donors in the present investigation. They were sensitized 9 to 12 weeks prior to use by the intraperitoneal injection of approximately 3 to 5 mg. of living *Mycobacterium tuberculosis* var. *bovis* of the BCG strain. Only animals showing strong reactions to the intradermal injection

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[2] This investigation was supported, in part, by a research grant from the National Institutes of Health, Public Health Service.

[3] Medical Research Fellow of the National Tuberculosis Association.

of 0.1 ml. of Old Tuberculin (OT)[4] diluted 1:100 were used as cell donors. Granulocytic exudates were induced in the donor animals by the intraperitoneal injection of 8 to 10 ml. of nutrient broth. The animals were killed 3 to 4 hours later. Their peritoneal cavities then were washed out with heparinized guinea pig serum Tyrode solution and the cells collected and washed by centrifugation in fresh solution. For each transfer the cells of 5 to 7 donor animals were pooled, suspended in heparinized serum Tyrode solution and injected intraperitoneally into a normal, large, male, albino guinea pig. The cellular composition and the viability of the cells in the suspensions were determined by the neutral red supravital staining technique of Sabin (10). Approximately 90 per cent of the cells were alive and consisted almost exclusively of granulocytes. Forty-eight to 65 hours following the cell transfer, the tuberculin sensitivity of the recipient animals was tested by either intradermal injection of 0.1 ml. of OT diluted 1:5 or by the intraperitoneal injection of 2 ml. of OT diluted to 10 ml. with normal saline. Two transfers with monuclear cell suspensions obtained from tuberculin-sensitive guinea pigs by the method of Chase (1) also were carried out in order to

TABLE 1

Results of Cutaneous or Systemic Tuberculin Challenge of Guinea Pigs 48 to 65 Hours Following Transfer of Cells from Tuberculous Guinea Pigs

NUMBER OF TRANSFERS MADE	TYPE OF CELLS TRANSFERRED	SENSITIZING DOSE OF CELLS IN ML.	DOSE AND ROUTE OF INJECTION OF OT	CUTANEOUS OR SYSTEMIC REACTION OF RECIPIENT ANIMAL
6	granulocytes	1.0–1.5	0.1 ml. OT 1:5 intracutaneously	negative
1	mononuclear cells	1.0	0.1 ml. OT 1:5 intracutaneously	3+ tuberculin reaction
4	granulocytes	1.2–1.5	10.0 ml. diluted OT intraperitoneally	no symptoms
1	mononuclear cells	1.0	10.0 ml. diluted OT intraperitoneally	died at 24 hours

confirm that the degree of sensitivity developed by the donor animals was sufficient to render their mononuclear cells suitable for passive transfer. The 2 animals receiving these suspensions were tested respectively by the intracutaneous or intraperitoneal injection of OT in a manner identical with the one described previously.

RESULTS

The results of the experiments are summarized in table 1 and show that the recipients of viable granulocytes from tuberculin-sensitive guinea pigs did not respond to either cutaneous or systemic challenge with tuberculin 48 to 65 hours following the transfer. Cutaneous injection of 0.1 ml. of OT (1:5) caused a typical delayed skin reaction in the animal which had received, 48 hours prior to testing, an intraperitoneal injection with a mononuclear cell suspension from tuberculin-sensitive donor animals. The other recipient of such a cell suspension developed severe symptoms of tuberculin shock 4 hours following the challenging injection of tuberculin and died from tuberculin shock at 24 hours. Additional attempts to elicit cutaneous reactions to tuberculin in guinea pigs 24, 72, and

[4] The Old Tuberculin used in these experiments was kindly supplied by Parke, Davis & Company, Detroit, Michigan.

96 hours following the transfer of granulocytes from tuberculin-sensitive guinea pigs also were unsuccessful.

COMMENT

Kirchheimer and Weiser (3) have postulated that in the case of mononuclear cells the transferred sensitivity may be due to antibody elaborated by the donor cells during their residence in the recipient rather than to preformed antibody. If this were true, it would tend to explain why it is not possible to transfer the sensitivity with cells, such as granulocytes, which are not considered as being involved in the elaboration of antibody[5] (11).

It has been shown recently (12) that lymphocytes of tuberculin-sensitive humans and guinea pigs may liberate a factor active in the *in vitro* lysis of granulocytes by tuberculin. It would be interesting to know if this factor is identical with the one involved in the transfer of tuberculin-type sensitivity. That this might be the case is suggested by the observation of Sandage and Birkeland (13) who succeeded in passively sensitizing normal rabbits with blood taken from tuberculous rabbits shortly following an intravenous injection of tuberculoprotein. This injection might have caused lysis of lymphocytes resulting in the liberation of the sensitizing agent into the blood stream.

SUMMARY

Attempts were made at homologous passive transfer of tuberculin-type sensitivity with living granulocytes of tuberculin-sensitive guinea pigs. The results of the experiments show that it was not possible to elicit either cutaneous or systemic tuberculin reactions in the animals which received granulocytes from tuberculin-sensitive guinea pigs.

SUMARIO

Tentativas de Transferencia Pasiva de Sensibilidad Tuberculinoidea con Granulocitos Vivos

Hiciéronse esfuerzos para obtener transferencia pasiva homóloga de sensibilidad tuberculinoidea con granulocitos vivos de cobayos tuberculinosensibles. Los resultados de los experimentos demuestran que no fué posible evocar reacciones tuberculínicas ni cutáneas ni generales en los animales que recibieron granulocitos de cobayos tuberculinosensibles.

RESUME

Tentative de transfert passif de la sensibilité "type tuberculine" à l'aide de granulocytes vivants

Des essais de transfert passif homologue d'une sensibilité d'ordre tuberculinique ont été effectués avec des granulocytes vivants provenant de cobayes sensibilisés à la tuberculine. Les résultats de l'expérimentation montrent qu'il n'a pas été possible de provoquer des réactions d'ordre tuberculinique soit

[5] Elaboration of antibody here refers only to actual synthesis of antibody by the cell.

cutanées, soit générales, chez l'animal ayant reçu des granulocytes de cobayes sensibilisés à la tuberculine.

REFERENCES

(1) Chase, M. W.: Cellular transfer of cutaneous hypersensitivity to tuberculin, Proc. Soc. Exper. Biol. & Med., 1945, *59*, 132.

(2) Cummings, M. M., Hott, M., and Gottshall, R. Y.: Passive transfer of tuberculin sensitivity in the guinea pig, Pub. Health Rep., 1947, *62*, 994.

(3) Kirchheimer, W. F., and Weiser, R. S.: The tuberculin reaction: I. Passive transfer of tuberculin sensitivity with cells of tuberculous guinea pigs, Proc. Soc. Exper. Biol. & Med., 1947, *66*, 166.

(4) Kirchheimer, W. F., and Weiser, R. S.: The tuberculin reaction: III. Transfer of systemic tuberculin sensitivity with cells of tuberculous guinea pigs, Proc. Soc. Exper. Biol. & Med., 1949, *70*, 99.

(5) Stavitzky, A. B.: Passive cellular transfer of the tuberculin type of hypersensitivity, Proc. Soc. Exper. Biol. & Med., 1948, *67*, 225.

(6) Metaxas, M. N., and Metaxas-Buhler, M.: Über passive lokale Tuberkulinallergie, Schweiz. Ztschr. f. Path. u. Bakt., 1948, *11*, 414.

(7) Lawrence, H. S.: The cellular transfer of cutaneous hypersensitivity to tuberculin in man, Proc. Soc. Exper. Biol. & Med., 1949, *71*, 516.

(8) Prausnitz, C., and Küstner, H.: Studien über die Überempfindlichkeit, Centralbl. f. Bakt., 1921, *86*, 160.

(9) Minor, A. H., and Burnett, L.: Method for obtaining living leukocytes from human peripheral blood by acceleration of erythrocyte sedimentation, Blood, 1948, *3*, 799.

(10) Sabin, F. R.: Studies on living human blood cells, Bull. Johns Hopkins Hosp., 1923, *34*, 277.

(11) Bunting, C. H.: The Polymorphonuclear Neutrophile Leucocyte, Handbook of Hematology, vol. I, Paul B. Hoeber, Inc. New York, 1938.

(12) Miller, J. M., and Favour, C. B.: Lymphocytic origin of a plasma factor responsible for *in vitro* tuberculin-type hypersensitivity, Federation Proc., 1950, *9*, Part I, 388.

(13) Sandage, C. N., and Birkeland, J. M.: Passive transfer of tuberculin hypersensitivity with blood taken from infected rabbits during the response to tuberculin, Am. Soc. Bact., General Meeting (abstracts of papers), Cincinnati, 1949, *49*, p. 74.

THE RELATIONSHIP BETWEEN THE VIRULENCE OF TUBERCLE BACILLI AND THE VIGOR OF THEIR OXIDATIVE ATTACK UPON CERTAIN SUBSTRATES[1,2]

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INTRODUCTION

Although it may be assumed that virulence of pathogenic microorganisms is in some way referable to the physiology of the parasite and of the host, there is very little information on the biochemical mechanisms involved. Virulent strains of *Mycobacteria* presumably owe their virulence to their increased capacity to withstand the defensive mechanisms of the body; but it also seems likely that virulent *Mycobacteria* are better able than are the nonvirulent strains to meet their physiologic requirements in the host tissues and that virulence is associated with the biochemical reactions involved in the growth of the pathogen.

The present report deals with studies on the relation of virulence of tubercle bacilli to their oxidative attack on certain substrates which are present in host tissues and which can serve as a source of energy and material for growth.

In 1933 Loebel, Shorr, and Richardson (1) demonstrated manometrically that resting cells of the H37 strain of human tubercle bacillus are capable of oxidizing DL lactic acid in physiologic concentrations and, although they were unable to obtain growth in a medium containing 5 per cent lactic acid, they did observe a slight but consistent increase in the size of a bit of pellicle floated upon a medium containing 1 per cent of the acid. They concluded, therefore, that the lactate present in the host's blood and leukocytes "may furnish energy for the survival of the bacteria." More recently, this suggestion has been supported by a number of findings. Schaefer (2) has succeeded in growing virulent human tubercle bacilli in a Dubos medium with lactate as the sole source of carbon. Edson (3) prepared a cell-free extract of *Mycobacterium phlei* which vigorously oxidized L(+) lactic acid, the physiologic isomer. The present writers (4) have been able to prepare similar extracts from *M. smegmatis* and from the H37 strain of human tubercle bacillus. We have also found that the pH optimum of the cell-free mycobacterial L(+) lactic acid dehydrogenase is at least as low as 6.5.

This last finding is especially provocative for a number of reasons. First, leukocytes possess aerobic as well as anaerobic glycolytic mechanisms (5, 6, 7) of sufficient activity that the lactic acid content of cellular exudates may rise to as much as twenty times the blood concentration (8). Second, the pH of an inflamed area falls below the blood level of 7.4, sometimes dropping to as low as

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[3] Present address: Camp Detrick, Frederick, Maryland.

5.9 (6, 9, 10). Finally, in leukocytes, within which virulent tubercle bacilli are known to multiply, the internal pH has been shown to be quite low (11, 12, 13), especially in the vicinity of ingested cells (14, 15). It seemed worth while, therefore, to see whether the pathogenic *Mycobacteria* are better adapted than are the nonpathogenic to benefit from the lactate present in the tissues, especially under the acid conditions occurring in inflamed areas and within phagocytes of a potential host. Accordingly, a comparison has been made between the L(+) lactic acid oxidation by human tubercle bacilli and the L(+) lactic acid oxidation by various other *Mycobacteria* at various levels of pH.

MATERIALS AND METHODS

Test organisms: Throughout this report the term *Mycobacterium* is used as defined in Bergey's Manual (21), the term *tubercle bacillus* is applied to any mycobacterial strain pathogenic for animals and also to any avirulent strain derived therefrom, and the term *saprophyte* is applied to all *Mycobacteria* that are not tubercle bacilli. In parts I and II the strains employed included *Mycobacterium tuberculosis* var. *hominis* strain H37[4], the pathogenic human strain used by Loebel and associates (1) and moderately virulent for guinea pigs; *M. avium*, isolated from an infected chicken at The Ohio State University in 1943 and highly virulent for fowl; *M. smegmatis* (ATCC 101)[5]; *M. phlei* (ATCC 354)[5]; *M. tuberculosis* (ATCC 607)[5], which is avirulent and apparently a saprophyte; and BCG (ATCC 8420)[5]. The last strain resembles the saprophytes in its growth characteristics more than it does other BCG strains with which we have since worked.

The following strains of human tubercle bacilli were employed in part III and are part of the collection of Dr. René J. Dubos of The Rockefeller Institute for Medical Research: Amesaga[6], a strain of high virulence, H37Rv[7], a strain which was highly virulent when first received. The culture of the latter strain maintained in Tween-albumin medium (16) at The Rockefeller Institute became somewhat attenuated within the last year, while a culture of it supplied by Doctor Dubos to Dr. Hubert Bloch of the Public Health Institute of the city of New York and kept on the same medium has maintained its full virulence. We shall call the fully virulent strain H37Rv (Bloch) and the slightly attenuated strain H37Rv (Dubos). H37Ra[7], a completely avirulent mutant of H37Rv isolated from the original H37 culture (17, 18), and RIRv[7], a strain which causes lesions in Rockefeller Swiss albino mice (18) and in guinea pigs but seldom kills them unless they be silicotic (19), are also part of the Dubos collection. The latter strain is slightly less virulent than H37Rv (Dubos) which causes fatal although slowly progressing infections in R.S.a. mice (20).

Culture methods and media: The organisms investigated in parts I and II of this study were usually grown at 37°C. on Petragnani slants until maximum growth was attained. The length of time needed for this varied from strain to strain; *M. smegmatis* and *M. phlei* achieved full growth in less than four days and H37 required between one and two weeks for a maximum crop. Experiments performed with cultures of each strain at different ages showed that the differences between the results within a strain were small compared with the differences observed between different strains.

The organisms used in part III were grown on oleic acid-albumin agar or in liquid Tween-albumin medium (16) at 37°C. for varying lengths of time.

Preparation of suspensions: Cells grown on solid media were suspended by the bead-flask

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[4] Received in 1943 from The Henry Phipps Institute, Philadelphia, Pennsylvania.

[5] Received in 1946 from the American Type Culture Collection, Washington, D. C.

[6] Received in 1948 from Dr. J. D. Aronson of The Henry Phipps Institute, Philadelphia.

[7] Received in 1946 from the National Tuberculosis Standard Culture Depot, Trudeau, New York.

technique. A mass of cells was introduced into a sterile, dry, 500 ml. Erlenmeyer flask which contained a layer of glass beads 2 to 3 cm. in depth. The flask was rotated until the cells were evenly spread over its inner surface and over the beads. Suspending fluid was added and the flask was rotated again until the growth was washed off the beads and the flask wall to give a homogeneous suspension. The test organisms grew on the Petragnani slants in raised heaps, and most of the growth could be lifted off with no scraping of the medium. These cells were suspended without washing. However, the cells grown on the oleic acid-albumin agar had to be harvested by scraping and were washed once by centrifugation in 20 ml. of diluent for each ml. of packed cells before suspending. The resulting suspensions generally contained 0.6 mg. of bacterial nitrogen per ml.

The cells grown in liquid medium were centrifuged and washed once by centrifugation in 10 ml. of diluent (see below) for each 250 ml. of original culture. They were then resuspended by adding 1.5 ml. of diluent for each 250 ml. of original culture to the centrifuge bottle and shaking vigorously. The resulting suspensions were rather unevenly dispersed, and a special mode of analyzing the data was necessary (see part III). The Kjeldahl nitrogen content of these suspensions varied and will be reported for each suspension with the experimental results.

Suspending media and reagents: The suspensions used in parts I and II were made with 0.01 M pH 7.4 Sorensen phosphate buffer containing 0.5 per cent NaCl. The suspensions used in part III were made with a diluent containing the mineral ingredients of a Dubos basal medium in the following proportions: 0.05 gm. $FeCl_3$; 0.01 gm. $MgSO_4.7H_2O$; 0.0005 gm. $CaCl_2$; 0.0001 gm. $ZnSO_4$; 0.0001 gm. $CuSO_4$; 50 ml. M/15 KH_2PO_4; 50 ml. M/15 Na_2HPO_4; 900 ml. distilled water. All the inorganic salts and other readily available compounds were of reagent or analytical grade, except the propionic and butyric acids, which were CP.

The experiments reported here were performed using either Merck's "Acid Lactic-Reagent" or Pfanstiehl's 40 per cent solution of the sodium salt of L(+) lactic acid. Preliminary and corroborative experiments were also done using the zinc salt of L(+) lactic acid of which we had two samples, one obtained from Pfanstiehl and one prepared by Doctor Thompson of the Chemistry Department of The Ohio State University.

Standard Warburg manometric techniques for the measurement of oxygen consumption were employed throughout. The gas phase was air. The CO_2 produced was absorbed with 0.2 ml. of 10 per cent NaOH in the center well of the Warburg vessel.

RESULTS

I. *The L(+) Lactic Acid Dehydrogenase Activity of Mycobacteria and the Effect of pH upon This Activity*

One strain of *Mycobacterium* was studied in each experiment. Five substrate-containing vessels and three endogenous controls were set up. One and five-tenths milliliters of a suspension of Petragnani-grown cells and 0.8 ml. of 0.08 M phosphate buffer were pipetted into each of the eight vessels. The pH of the buffer added to each vessel was such that the final pH of the substrate-containing flasks ranged from 6.2 to 7.5, and the final pH of the endogenous controls was 6.2, 6.8, and 7.5. The substrate was 0.2 ml. of a 1 per cent solution of sodium L(+) lactate added to the side arms of the appropriate vessels and in the endogenous controls 0.2 ml. of distilled water was substituted. The vessels were equilibrated for twenty minutes at 37°C., then the contents of the side arms were tipped in with the manometer stopcocks open, and the vessels were further equilibrated for ten more minutes before the stopcocks were closed and readings begun. After the experiments were completed, the final pH was determined with a Coleman glass-electrode pH meter, except in the case of the H37 suspensions for which a Helliger comparator was used.

The results of typical experiments with strain H37 and with *M. phlei* are listed in table 1 as samples of the data. The values obtained with BCG and strain 607 and *M. smegmatis*, the other bacteria studied, fell between the values for the two strains given in the table.

The results of the complete study are shown in figure 1 and are expressed in terms of the ratio, uptake with lactate divided by uptake without substrate,

TABLE 1

The Influence of L(+) Lactic Acid upon the Oxygen Consumption of Resting Mycobacterial Suspensions at Various pH's

FINAL pH	µl. OXYGEN TAKEN UP IN ONE HOUR BY 1 MG. BACTERIAL NITROGEN			
	M. tuberculosis var. *hominis* strain H37		*M. phlei*	
	No addition	0.01 per cent Na lactate	No addition	0.01 per cent Na lactate
7.5	18.7	25.4	23.6	123
7.2	—	27.7	—	131
6.8	19.5	34.3	20.7	146
6.5	—	41.1	—	164
6.2	21.1	41.7	17.6	271

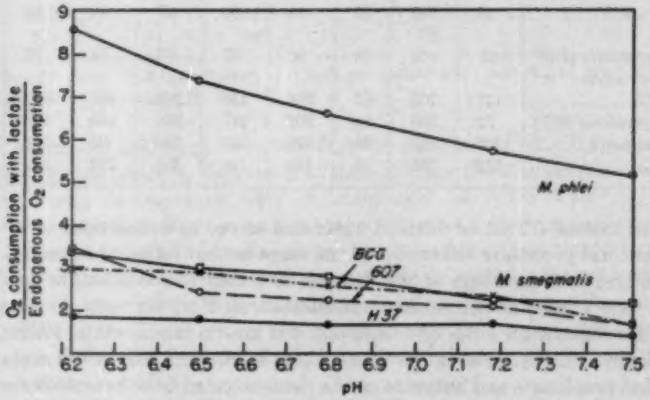

FIG. 1. The influence of L(+) lactic acid upon the oxygen consumption of mycobacterial suspensions at various levels of pH.

plotted against final pH. Each point represents the average of values obtained in two experiments.

It can be seen that, contrary to our initial expectations, there was, among the mycobacterial strains tested, a negative correlation between pathogenicity and the vigor of their oxidative attack upon lactate. These unexpected results seemed to warrant further investigation, and the first step taken was to ascer-

tain whether this negative correlation holds only with lactate or with other substrates as well.

II. *The Correlation Between Pathogenicity and the Stimulation of Oxygen Uptake by Short-Chain Fatty Acids*[6]

In addition to the five mycobacterial strains employed in the preceding section, a strain of *M. avium*, highly virulent for chickens, was used in the experiments that follow. Cells grown on Petragnani slants were suspended in 0.01 M pH 7.4 phosphate buffer in the manner described. A volume of 2.3 ml. of suspension was added to each Warburg vessel. To the side arms of all but one of the vessels used in an experiment were added 1 mg. portions of carefully neutralized substrate in 0.2 ml. of water. The vessel not receiving any substrate

TABLE 2

Effect of Lower Fatty Acids upon Oxygen Consumption by Various Mycobacterial Strains

Organism	Endogenous oxygen consumption (μl/1.5 hr)	0.04 per cent formic		0.04 per cent acetic		0.04 per cent propionic		0.04 per cent butyric	
		Oxygen consumption μl/1.5 hr)	Increase over endogenous (per cent)	Oxygen consumption (μl/1.5 hr)	Increase over endogenous (per cent)	Oxygen consumption (μl/1.5 hr)	Increase over endogenous (per cent)	Oxygen consumption (μl/1.5 hr)	Increase over endogenous (per cent)
M. avium	48	65	35	74	54	67	40	66	37
M. tuberculosis (H37)	48	72	50	80	67	74	54	76	58
M. tuberculosis (BCG)	121	203	67	304	150	243	100	217	79
M. tuberculosis (607)	72[7]	103	81	202	181	208	180	166	130
M. smegmatis	102	161	58	298	192	199	95	190	86
M. phlei	152	251	65	450	196	339	123	302	99

received instead 0.2 ml. of distilled water and served as endogenous control. The experimental procedure followed was the same as that followed in part I.

The first four members of the normal saturated monocarboxylic fatty acid series were chosen as convenient test substances. For, not only do fatty acids occur throughout all kinds of tissue, but it is known that acetate, although not chemically demonstrable in the blood, plays a cardinal role in cell metabolism and that propionate and butyrate can be demonstrated to be present in ruminant blood (22). The results of typical experiments with each of the test organisms are shown in table 2.

As may be seen in table 2, with the fatty acids also, the oxygen consumption of the two unquestionably pathogenic strains was stimulated less by the added substrates than was the case with the other four *Mycobacteria*. Of the two patho-

[6] We are greatly indebted to Dr. Clarke T. Gray (present address, Leonard Wood Memorial, Department of Bacteriology and Immunology, Harvard University Medical School, Boston) for his collaboration in this part of our study.

genic strains, the highly virulent avian strain was even less vigorous in its substrate attack than was H37 which is only moderately virulent. It is beyond the scope of this paper to analyze in detail the difference between strains for each of these fatty acids. A comprehensive treatment has been given to this subject by Gray (23). He investigated the same six mycobacterial strains, and his data, gathered to shed light upon problems other than the one discussed here, show that the same activity relationship holds for every common fatty acid up to eighteen carbon atoms in length.

In the present study it was possible to demonstrate that the rate of oxidation by mycobacterial suspensions of propionate and butyrate, as well as lactate, was increased as the pH was lowered from pH 7.4 to pH 6.2. Similar results have been reported in the oxidation of acetate by virulent bovine (24) and human (25) tubercle bacilli. The data in table 3 from a typical experiment with butyric acid and *M. smegmatis* seem to indicate that, to obtain the pH effect, the concentra-

TABLE 3

*The Influence of pH and Concentration upon the Oxidation of Butyric Acid by Resting Cell Suspensions of M. Smegmatis**

FINAL pH	μl. OXYGEN TAKEN UP IN 30 MINUTES BY 1 MG. BACTERIAL NITROGEN		
	No addition	0.006 per cent butyric acid	0.003 per cent butyric acid
6.2	26	84	80
7.4	25	78	62

* Grown for three days on nutrient agar and suspended after washing in 0.013 M phosphate buffer of appropriate pH.

tion of the organic acid must be so low that the amount of acid getting into the cell is the limiting factor in the rate of oxidation. Since the dissociation constant of butyric acid is one-tenth that of lactic acid ($K = 1.5 \times 10^{-5}$ vs. $K = 1.4 \times 10^{-4}$), there is present, in an aqueous solution of a given molarity at any given pH, ten times as much undissociated butyric acid as there would be lactic acid. The fact that the molar concentration of butyric at which the pH begins to become noticeable is of the order of magnitude of one-tenth the molar concentration of L(+) lactate at which the pH effect can be demonstrated suggests that organic acids such as those employed in this study enter the mycobacterial cell as undissociated acid.

III. *The Correlation Between Virulence and Lactic Acid Oxidation Within the Group of Tubercle Bacilli from Human Sources*

Evidence has been obtained that there was a marked difference in the extent to which pathogenic and saprophytic *Mycobacteria* were stimulated in their oxygen consumption by lactate and the lower fatty acids. It was necessary, therefore, to ascertain whether there was similarly, among the pathogens alone, a negative correlation between the vigor of oxidative attack and degree of viru-

lence. At this point, we were fortunate in having made available to us by Dr. René J. Dubos of The Rockefeller Institute for Medical Research his culture collection of tubercle bacilli and the facilities of his laboratory.

For the experiments on this point, lactate was once again used as a substrate. The final concentration of L(+) lactic acid in each vessel was 17 mg. per 100 cc., calculating from a DL lactic acid content of 85 per cent in Merck's reagent lactic acid. This is close to the median value reported for resting venous human blood in fourteen separate investigations (26). As test organisms the five strains of human tubercle bacilli supplied by Doctor Dubos were employed. Four of these strains, the highly virulent Amezaga and H37Rv (Bloch), the slightly less virulent H37Rv (Dubos), and the somewhat attenuated R1Rv could not be distinguished with any degree of certainty from each other by existing *in vitro* methods. The fifth strain, H37Ra, was included because it is an avirulent mutant of H37Rv. This strain is very easily distinguished from the other four strains by manner of growth and morphology (18) and by the neutral red test (27). In addition to the human tubercle bacilli listed above, either *M. phlei* or *M. smegmatis* was included in a number of our experiments for the purpose of comparison.

Experiments were performed with cultures grown both on liquid and solid media. Furthermore, both young and relatively old cultures were used to ascertain whether findings made in some preliminary experiments possessed any degree of generality. The cells of all the strains used in any single experiment were grown for the same length of time under the same conditions on the same batch of medium.

It was difficult to grow enough cells in the liquid medium to obtain a large oxygen uptake. Furthermore, the suspensions prepared from these cells were not as homogeneously dispersed as those prepared by the bead-flask method. Consequently, there was reason to fear that the small differences in metabolic behavior that could be expected between closely related strains might be hidden by experimental error. To reduce this uncertainty, the following technique was employed:

Two Warburg vessels, each containing 2.0 ml. of a cell suspension plus 0.3 ml. of 0.13 M pH 6.8 phosphate buffer, were set up with each strain tested. The side arm of one of these vessels contained 0.2 ml. of neutralized lactate solution, the side arm of the other contained 0.2 ml. of distilled water. The vessels were equilibrated for two hours in the water bath at 37°C., and then the stopcocks were closed and readings taken. At the end of ninety minutes the contents of the side arms were tipped with the manometers closed, and the readings continued for another ninety minutes. What may be called a "ratio of stimulation" was computed thus:

$$\frac{\dfrac{\text{uptake substrate flask after tipping}}{\text{uptake control flask after tipping}} + \dfrac{\text{uptake substrate flask before tipping}}{\text{uptake control flask before tipping}}}{}$$

By subtracting unity from this value and multiplying by 100, the per cent stimulation could be computed. In this manner it was possible to eliminate the error due to the inequality in the numbers of cells in the different vessels and to make the results independent

of the accuracy of the calibration of the vessels and also independent of any falling off of the endogenous rate after tipping.

The results of five experiments are given in table 4. These data showed that the negative correlation between vigor of lactate oxidation and virulence held

TABLE 4

Stimulation by Lactate of Oxygen Consumption by Human Tubercle Bacilli

STRAIN*	MILLIGRAMS KJELDAHL N PER VESSEL	μl. OXYGEN CONSUMED IN 90 MINUTES PER VESSEL				PER CENT STIMULATION‡
		Control vessel		Lactate† vessel		
		Before tipping	After tipping	Before tipping	After tipping	
(a) 9-day Tween-albumin cultures						
H37Rv (Bloch)	1.0	85	61	88	80	27
H37Rv (Dubos)	0.6	65	47	67	83	71
(b) 9-day Tween-albumin cultures						
Amezaga	0.7	88	51	86	63	30
R1Rv	0.8	137	87	132	118	41
(c) 12-day Tween-albumin cultures						
H37Rv (Dubos)	0.7	54	35	48	42	35
R1Rv	0.7	87	74	88	97	29
H37Ra	0.5	60	52	70	96	58
M. smegmatis§	0.4	21	22	15	60	280
(d) 14-day Tween-albumin cultures						
Amezaga	0.7	43	38	38	41	22
R1Rv	1.1	48	42	48	69	64
H37Ra	0.8	36	35	36	63	80
M. phlei§	0.6	23	19	19	61	290
(e) 18-day oleic acid-albumin agar cultures						
H37Rv (Bloch)	1.1	50	41	43	51	45
H37Rv (Dubos)	1.1	54	48	50	79	77

* Strains employed in each experiment are listed in order of decreasing virulence.
† 34 mg. per cent DL lactic acid neutralized with NaOH.
‡ Computed as in text.
§ Saprophytes.

for even such closely related forms as the particular strains of human tubercle bacilli tested.

The question arises whether the metabolic phenomena observed here by manometric methods have any parallels observable in growth studies. Dubos (28, 29) has shown that such parallels do exist. Since Dubos' studies were done at the same time in the same laboratory and included the same cultures which

were employed in the experiments summarized in table 4, the data reported by Dubos are very well suited to be discussed together with the data reported in the present paper.

DISCUSSION

It is evident from the data presented above, as well as from the literature, that the oxygen consumption of resting cell suspensions of *Mycobacteria* is strongly stimulated by certain physiologically occurring organic acids. Moreover, this stimulation becomes more marked as the pH of the suspensions is lowered from pH 7.4 to approximately pH 6. In his experiments Dubos (28) has shown that similar concentrations of these same acids strongly inhibit the multiplication of tubercle bacilli and that this inhibitory effect becomes more marked when the pH of the medium is lowered from pH 7 to pH 6.

In the present study, it has also been shown that, in the general group of *Mycobacteria*, saprophytes oxidize lactic and low molecular-weight fatty acids more rapidly than do pathogens and that, in the more restricted and homogeneous group of human tubercle bacilli, the less virulent strains oxidize lactic acid more rapidly than do the more virulent strains. In parallel, Dubos (29) has found that, among both human and bovine tubercle bacilli, less virulent strains are more susceptible to inhibition by short-chain organic acids than are more virulent strains. For example, only 45 mg. per 100 cc. of DL lactic acid sufficed for a 50 per cent inhibition of the growth of R1Rv, while 72 mg. per 100 cc. of the acid was required for a similar inhibition of H37Rv (Bloch).

Thus it can be seen that, on the one hand, a correlation exists between the degree of virulence of tubercle bacilli and the concentrations of lower organic acids which they can tolerate and, on the other hand, there is a correlation between the degree of virulence of the bacilli and the slowness with which they oxidize these acids. Consequently, it is possible that the inhibition of the growth of tubercle bacilli by lactic and the other physiologically occurring acids studied is related to the degree of stimulation of the bacteria by the acids. If this were true, it would follow that one of the necessary conditions that must be satisfied before a given strain of tubercle bacillus were able to invade a given host successfully would be that the parasite should not oxidize certain substances in the host tissues too rapidly. This condition conceivably could be satisfied either by a metabolic conservatism on the part of the invader or by a relatively low concentration of the significant metabolites in the host tissues.

It is well known that tubercle bacilli are capable of oxidizing a great number of substrates (including hydrocarbons, alcohols, aldehydes, fatty acids, amino acids, aromatic acids, carbohydrates, phospholipids, and diamines) and are capable of using many of them as carbon and energy sources for growth. Moreover, enormous yields of the most virulent strains of tubercle bacilli are routinely obtained in the laboratory upon simple media containing only mineral salts, ammonia, a carbon and energy source such as glucose or glycerol, and a source of four carbon dicarboxyllic acid such as succinate. Since the nutritive requirements of the tubercle bacillus are so very simple and the substances present in a host

capable of satisfying these requirements are so abundant, the question arises whether the nutritive problem of an invading tubercle bacillus might not be one of excluding, rather than of obtaining, nutrients from the host.

Such a view finds some support from what is known about the mycobacterial cell and its metabolism. All other kinds of cells, as far as is known, normally are more restricted in the capacity of their preliminary carbon-chain desmolytic systems than in their terminal system, i.e., the tricarboxyllic acid cycle (30). In contrast, the limiting factor in the rate of carbon-chain desmolysis in the mycobacterial cell is the terminal system, which also appears to be a Krebs cycle (31). It would be expected, therefore, that various metabolic intermediates and their dismutation products would appear in a culture medium in which tubercle bacilli are growing. Such substances do occur both in the case of other kinds of cells respiring anaerobically and in the case of abnormal or aging tissue cells, such as cancer cells and exudate leukocytes, where the citric acid cycle does not keep pace with the preliminary carbon-chain desmolysis. Nevertheless, although many attempts have been made to find them, no products of carbon source dissimilation other than carbon dioxide have been detected in cultures of tubercle bacilli. The interpretation which is generally given to this last phenomenon is that tubercle bacilli do not form the various incompletely oxidized compounds commonly found in cultures of other bacteria. It is possible, however, that intermediates and their dismutation products are not found in the culture medium because they cannot readily pass out from the tubercle bacillus.

If this were the case, in the presence of an excess of certain oxidizable substrates, there might arise within the tubercle bacillus a condition very roughly analogous to what has long been known to occur within a deep culture tube in which *Escherichia coli* is growing anaerobically in the presence of an excess of fermentable sugar, i.e., an accumulation of incompletely oxidized metabolic products which makes the milieu lethal. The inhibition of the growth of the tubercle bacillus seems to be, to judge from Dubos' data (28), not so much a case of the accumulation of frankly lethal products as it is a case of the satisfaction of equilibria and the saturation of carrier systems.

The mechanism suggested above might explain Schaefer's observation (2, 32) that acetate, propionate, and DL lactate can each be used as the sole source of carbon and energy by virulent human tubercle bacilli as long as the substances are supplied in concentrations no larger than approximately 0.1 per cent. Supermaximal concentrations, as confirmed by Dubos' work, are inhibitory. The proposed mechanism might also explain why tubercle bacilli are obligate aerobes (33); for it is clear that inhibition of the growth of tubercle bacilli can be brought about by a depression, such as would accompany anoxia, of their already limited terminal desmolytic system as well as by an increase in the supply of, say, lactate.

There are also certain practices current in tuberculosis therapy and certain observations reported in the literature other than those given above which, while they are perhaps not admissible as support for this hypothesis, are indeed compatible with it. Thus the efficacy of forced feeding, rest, and collapse therapy, all

of which can be expected to result in either an increased concentration of certain oxidizable substrates or a lowered oxygen tension in at least some of the patient's tissues, may to some extent be due to the inhibition of even highly virulent tubercle bacilli by either an accumulation of a surplus of intermediate metabolites or a saturation of some carrier system or systems.

Finally, if the suggested hypothesis should be correct, it is possible that the differences in host specificity of different strains of virulent tubercle bacilli could be explained by the different kinds and amounts of substances, such as low molecular weight fatty acids which can be found in the tissues of different species of hosts. For example, ruminants absorb considerable quantities of fatty acids from their rumens so that there are demonstrable amounts of propionate and butyrate present in their blood (22), but no one has ever succeeded in demonstrating these substances in human blood. Moreover, Dubos has reported (28) that greater concentrations of acetic, propionic, and butyric acids, but not of lactic acid, are needed to inhibit the growth of the Ravenel strain, a tubercle bacillus highly virulent for cattle, than are needed to inhibit the growth of the H37Rv (Bloch) strain equally virulent for humans.

The hypothesis suggested in the above discussion represents an attempt to explain, in the light of present knowledge, the observed data which show that there is a negative correlation between the virulence and the vigor of oxidative attack in tubercle bacilli.

SUMMARY

Resting cells of the H37 strain of human tubercle bacillus, as well as of four other strains of *Mycobacteria*, have been shown to increase their oxygen consumption in the presence of L(+) lactic acid. The stimulation was found to increase as the pH of the suspending fluid was varied from pH 7.5 to pH 6.2. Stimulation was greatest, at any one pH, for the saprophyte *M. phlei* and was least for the pathogen H37.

Formate, acetate, propionate, and butyrate also increased the oxygen consumption of the five strains mentioned above, as well as that of a highly virulent avian strain. With these substances, also, the pathogens were least stimulated and the avian strain, known to be highly virulent, was less stimulated than the moderately virulent variant of the H37 strain.

With lactate in physiologic concentrations as substrate, it was possible, using stimulation of oxygen uptake as a criterion, to discriminate between highly virulent and attenuated strains of human tubercle bacilli otherwise indistinguishable from each other in every way except in their virulence for experimental animals. In this situation, also, there was a negative correlation between vigor of oxidative attack and degree of virulence.

It is suggested that one of the necessary conditions for virulence of tubercle bacilli might be the tendency to make a relatively weak attack upon certain oxidizable substances present in host tissues.

SUMARIO

La Relación entre la Virulencia de los Bacilos Tuberculosos y el Vigor de su Ataque Oxidante sobre Ciertos Subestratos

Ha sido demostrado que las células restantes de la cepa H37 del bacilo tuberculoso humano, así como de otras cuatro cepas de *Mycobacteria*, acrecientan su consumo de oxígeno en presencia de L (+) de ácido láctico. Observóse que esta excitación aumentaba a medida que se hacía variar el pH del líquido suspensivo, de 7.5 a 6.2. La excitación alcanzó su máximo a cualquier pH con el saprofito *M. phlei*, y su mínimo con el patógeno H37.

El formiato, el acetato, el propionato y el butirato también acrecentaron el consumo de oxígeno por las cinco cepas ya mencionadas, e igualmente por una virulentísima cepa aviaria. Aquí de nuevo, las patógenas fueron las menos excitadas, y la cepa aviaria, reconocidamente virulentísima, fué menos excitada que la variante moderadamente virulenta de la cepa H37.

Con el lactato a concentraciones fisiológicas como subestrato, fué posible, tomando como norma el alza de la absorción de oxígeno, separar cepas altamente virulentas y atenuadas de bacilos tuberculosos humanos absolutamente indiferenciables de otro modo, salvo por su virulencia en los animales de experimentación. Aquí, otra vez, hubo correlación negativa entre el vigor del ataque oxidante y el grado de virulencia. Discutidos los resultados, sugiérese que una de las condiciones necesarias para la virulencia de los bacilos tuberculosos acaso sea un ataque relativamente débil sobre ciertas substancias oxidables de los tejidos del huésped.

RESUME

La relation entre la virulence des bacilles tuberculeux et l'importance de leur pouvoir oxydant sur certains substrats

Les souches de virulence atténuée H37 de bacille tuberculeux humain, ainsi que quatre autres souches de *Mycobacteria* ont montré un accroissement de leur consommation d'oxygène en présence d'acide lactique L (+). Cette augmentation de la consommation d'oxygéne augmente lorsque le pH passe de 7,5 à 6,2; elle atteint son maximum, quel que soit le pH en présence du saprophyte *M. phlei*, tout au moins en ce qui concerne la souche virulente H37.

Les formiates, acétates, propionates et butyrates augmentent également la consommation d'oxygène des cinq souches mentionnées ci-dessus, au même titre que celle d'une souche aviaire de haute virulence. LÀ, également, la consommation d'oxygène des souches pathogènes a été moindre en présence d'une souche aviaire connue pour sa haute virulence, moindre également qu'une souche H37 de virulence moyenne.

Il a été possible, en se servant de lactate en concentration physiologique comme substrat, d'utiliser comme critère l'augmentation de la consommation d'oxygène pour établir une distinction entre les souches très virulentes et les

souches de virulence atténuée de bacilles tuberculeux humains, que d'autres méthodes, sauf l'expérimentation sur l'animal, ne permettent pas de discerner les unes des autres. Là, encore, l'importance de l'oxydation et le degré de virulence sont inversement proportionnels. Les résultats sont étudiés; les auteurs émettent l'hypothèse qu'une des conditions nécessaires à la virulence du bacille tuberculeux serait une attaque relativement faible réalisée sur certaines substances oxydables des tissus de l'hôte.

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REFERENCES

(1) Loebel, R. O., Schorr, E., and Richardson, H. B.: The influence of foodstuffs upon the respiratory metabolism and growth of human tubercle bacilli, J. Bact., 1933, *26*, 139.

(2) Schaefer, W. B.: Recherche sur la croissance du *Mycobacterium tuberculosis* en culture homogene, Ann. Inst. Pasteur, 1948, *74*, 458.

(3) Edson, N. L.: The oxidation of lactic acid by *Mycobacterium phlei*, Biochem. J., 1947, *41*, 145.

(4) Geronimus, L. H., Gray, C. T., and Birkeland, J. M.: Enzymic activity of cell-free extracts of *Mycobacteria*, Proc. Soc. Am. Bact. Meetings, 1949, 42.

(5) Fleischmann, W., and Kubowitz, F.: Ueber den Stoffwechsel der Leucocyten, Biochem. Ztschr., 1927, *181*, 395.

(6) Kempner, W., and Peschel, E.: Stoffwechsel der Entzündung, Zeitschr. klin. Med., 1930, *114*, 439.

(7) McLeod, J., and Rhoads, C. P.: Metabolism of leucocytes in Ringer-phosphate and in serum, Proc. Soc. Exper. Biol. & Med., 1939, *41*, 268.

(8) Barnett, G. D., and McKenney, A. C., Jr.: Lactic acid in exudates and transudates, Proc. Soc. Exper. Biol. & Med., 1926, *23*, 505.

(9) Schade, H., Neukirch, P., and Halpert, A.: Über lokale Acidosen des Gewebes und die Methodik ihrer intravitalen Messung, Ztschr. f. d. ges. exper. Med., 1921, *24*, 11.

(10) Menkin, V.: Studies on inflammation: X. The cytological picture of an inflammatory exudate in relation to its hydrogen ion concentration, Am. J. Path., 1934, *10*, 194.

(11) Rous, P.: The relative reaction within living mammalian tissues: I. General features of vital staining with litmus, J. Exper. Med., 1925, *41*, 379.

(12) Rous, P.: The relative reaction within living mammalian tissues: II. On the mobilization of acid material within cells and the reaction as influenced by the cell state, J. Exper. Med., 1925, *41*, 399.

(13) Ishikawa, A.: Die Wasserstoffionenkonzentration der Leucozyten, Nagoya J. Med. Sc., 1936, *10*, 257.

(14) Metchnikoff, E.: Immunity of Infectious Diseases, translated by F. G. Binnie, University Press, Cambridge, 1905, p. 181.

(15) Pulcher, C.: Le variazioni del pH nella facitosi: Nota II, Bol. soc. ital. biol. sper., 1927, *2*, 722.

(16) Dubos, R. J., and Middlebrook, G.: Media for tubercle bacilli, Am. Rev. Tuberc., 1947, *56*, 334.

(17) Steenken, W., Jr., and Gardner, L. U.: History of the H37 strain of tubercle bacillus, Am. Rev. Tuberc., 1946, *54*, 62.

(18) Middlebrook, G., Dubos, R. J., and Pierce, C. H.: Virulence and morphological characteristics of mammalian tubercle bacilli, J. Exper. Med., 1947, *86*, 175.
(19) Steenken, W., Jr., and Gardner, L. U.: R_1 strain of tubercle bacillus, Am. Rev. Tuberc., 1946, *54*, 51.
(20) Suter, E.: Unpublished data, 1950.
(21) Bergey's Manual of Determinative Bacteriology, ed. 6, Williams & Wilkins, Baltimore, 1948, p. 875.
(22) Barcroft, J., McAnally, R. A., and Phillipson, A. T.: The absorption of volatile acids from the alimentary tract of the sheep and other animals, J. Exper. Biol., 1944, *20*, 120.
(23) Gray, C. T.: The nature of the fatty acid metabolism of tubercle bacilli and other *Mycobacteria*, Ph.D. thesis, Ohio State University, 1949, 51.
(24) Bloch, H., and Suter, E.: Ueber den Stoffwechsel von Tuberkelbazillen, Schweiz. Ztschr. f. Path. u. Bact., 1946, *9*, 434.
(25) Bloch, H., Matter, E., and Suter, E.: The effect of glycerol and related substances on the growth and the oxygen uptake of the tubercle bacillus, Am. Rev. Tuberc., 1947, *55*, 540.
(26) Jervell, O.: Investigation of the concentration of lactic acid in blood and urine under physiologic and pathologic conditions, Acta med. Scandinav., 1938, Supplement *24*, 25.
(27) Dubos, R. J., and Middlebrook, G.: Cytochemical reaction of virulent tubercle bacilli, Am. Rev. Tuberc., 1948, *58*, 698.
(28) Dubos, R. J.: The effect of organic acids on mammalian tubercle bacilli, J. Exper. Med., 1950, *92*, 319.
(29) Dubos, R. J.: Bacteriological aspects of the pathogenesis of tuberculosis, J. Mt. Sinai Hosp. (in press).
(30) Krebs, H. A.: The intermediary stages in the biological oxidation of carbohydrates, Advances in Enzymol., 1943, *3*, 191.
(31) Geronimus, L. H.: Studies on oxidative enzyme systems in *Mycobacteria* with special emphasis upon the employment of cell-free extracts, Ph.D. thesis, Ohio State University, 1949.
(32) Schaefer, W. B.: Unpublished data.
(33) Novy, F. G., and Soule, M. H.: Microbic respiration: II. Respiration of the tubercle bacillus, J. Infect. Dis., 1925, *36*, 168.
(34) Soffer, L. J., Dantes, D. A., and Sobotka, H.: Utilization of intravenously injected d-lactate as a test of hepatic function, Arch. Int. Med., 1938, *62*, 918.

THE RELATION BETWEEN THE SIZE OF THE INFECTING DOSE OF TUBERCLE BACILLI AND THE SURVIVAL TIME OF MICE[1, 2]

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During the course of an investigation which had as its object the development of a standardized test for the determination of antituberculous activity of chemotherapeutic agents in mice (1), experiments were conducted to determine the effect of the size of the infecting dose of tubercle bacilli on the time of death of mice. In view of the interesting relationship found between the size of the infecting dose and the survival time, the results of these experiments are presented separately in the present report.

METHODS

The virulent human variety of *Mycobacterium tuberculosis*, strain H37Rv, was employed. Cultures of this organism were grown on the surface of a modified Proskauer and Beck medium and suspensions prepared and standardized as previously described (2). Mice of the "Strong A" strain, both male and female, and weighing between 18 and 22 gm. were employed for all experiments. They were infected by intravenous inoculation with doses of tubercle bacilli which varied between 0.1 and 12.0 mg. wet weight suspended in 0.4 ml. of 0.01 molar phosphate buffer, pH 7.0. The median survival times were calculated using the method of Litchfield (3).

RESULTS

The number of mice employed and the median survival times, as calculated from the mortality data, of the mice infected with doses of tubercle bacilli between 0.5 mg. and 12.0 mg. are shown in table 1. With doses of tubercle bacilli less than 0.5 mg., the distribution of deaths was not uniform. The median survival times in table 1 were calculated from the combined results of 2 to 5 different experiments in which 10 to 20 mice were employed for each infecting dose, with the exception of the median survival time of those mice infected with 1.0 mg. This value was obtained from the pooled data of the 28 separate experiments previously reported (1). The median survival times of replicate experiments with the other doses did not deviate significantly from one another so the data were pooled to give the results shown in table 1.

To exclude, if possible, vascular phenomena as a cause of death in the animals injected with the 10 mg. and 12 mg. doses of tubercle bacilli, several groups of mice were injected with these doses after the organisms had been killed with heat. In one experiment the tubercle bacilli were killed by heating at 60°C. for five hours, in another they were killed by autoclaving at twenty pounds' pressure for twenty minutes. In the case of those tubercle bacilli killed by heating at

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[2] This work was aided, in part, by a research grant from Parke, Davis & Company, Detroit 32, Michigan.

60°C. for five hours, none of the mice died following injection of the 10 mg. and 12 mg. doses. In the case of those mice injected with 10 mg. of tubercle bacilli killed by autoclaving, 7 of 20 mice died within six days, but the remaining 13 mice were alive thirty-seven days following the date of injection.

As a test of the thesis that mice injected with the larger doses of living tubercle bacilli actually died as the result of infection with the microorganisms and not because of some nonspecific effect, mice were infected with 4 mg. of tubercle bacilli and treated daily with 3.0 mg. of streptomycin sulfate for a period of fourteen days. The streptomycin-treated mice had a median survival time of 64.0 days as compared to the untreated mice which had a median survival time of 3.5 days. Another group of 20 mice was infected with an 8 mg. dose of tubercle bacilli and treated daily with 3.0 mg. streptomycin sulfate for fourteen days. Of this group, 12 mice (60 per cent) died within the first two days, that is, the same time period in which all of the 20 control animals died. The remaining 8 mice (40 per cent) lived ten to fifty-six days.

TABLE 1

Median Survival Times of Mice Infected Intravenously with Mycobacterium Tuberculosis var. Hominis (H37Rv)

INFECTING DOSE OF TUBERCLE BACILLI IN MG.	NUMBER OF MICE	MEDIAN SURVIVAL TIME (95 PER CENT CONFIDENCE LIMITS)
0.5	40	17.0 (16.4 -22.6)
1.0	467	14.0 (13.8 -14.2)
2.0	60	9.8 (9.3 -10.2)
3.0	40	5.2 (4.6 - 5.7)
4.0	80	3.5 (3.1 - 3.8)
6.0	40	1.7 (1.4 - 2.0)
8.0	20	0.26 (0.14- 0.49)
10.0	20	0.0
12.0	20	0.0

DISCUSSION

At first glance the results presented herein might appear only to confirm what is generally recognized, that is, that the survival time of mice infected intravenously with virulent tubercle bacilli is inversely proportional to the number of organisms injected. This relationship is represented graphically in figure 1. Analysis of the data, however, brings out several interesting points. When, as shown in figure 2, the logarithms of the infecting doses are plotted against the median survival times, a curve is obtained which approximates linearity. The demonstration of such a relationship raises several interesting possibilities.

For example, one can calculate from the data by the method of least squares the slope of the line shown in figure 2. When this was done, using the data from table 1, a value of 14.77 was obtained with a probable error of 0.565 (3.82 per cent). The reciprocal of this slope value $\left(\frac{1}{14.77}\right)$ equals 0.067 and gives the

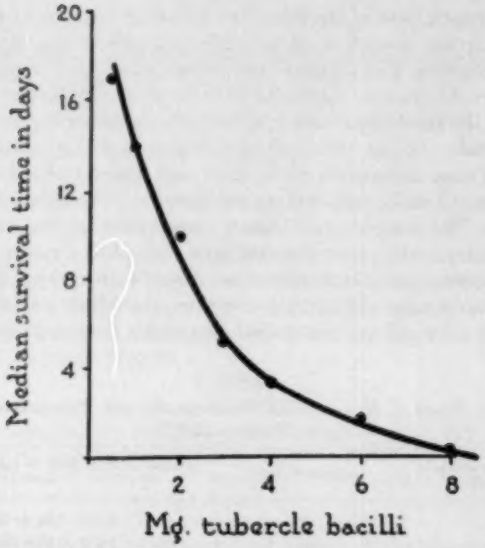

FIG. 1. The effect of the size of the infecting dose of *M. tuberculosis* var. *hominis* (H37Rv) on the median survival time. Arithmetic plot.

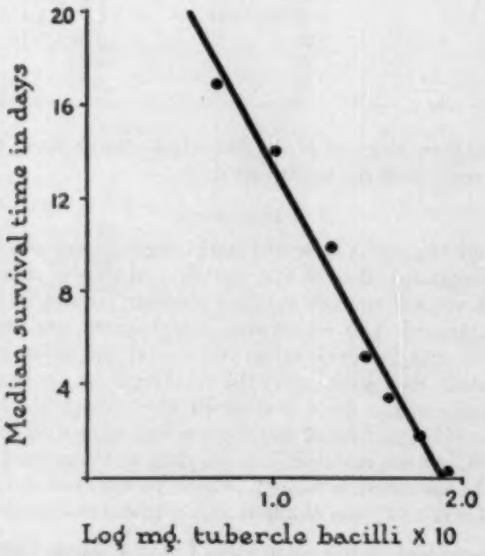

FIG. 2. The effect of the size of the infecting dose o *M. tuberculosis* var. *hominis* (H37Rv) on the median survival time. Semilog plot.

value for the slope in terms of the logarithm of the number of tubercle bacilli injected. The significant feature is that this value may represent the rate of growth of the tubercle bacilli in the mice.

The reason for the above statement probably can be best illustrated by drawing an analogy between these experiments in mice and those done *in vitro* to determine the rate of growth of tubercle bacilli by the small inocula method (4). When using this method, tubes of culture medium are inoculated with different numbers of tubercle bacilli, allowed to incubate, and the time which is required for each inoculum to attain a certain mass is determined. If the tubercle bacilli grow at the same rate in all tubes regardless of the size of the inoculum, when the logarithm of the number of tubercle bacilli inoculated into each set of tubes is plotted against the time required for the tubercle bacilli in each set of tubes, respectively, to attain a certain mass, a linear relationship will be found. It can be readily shown (4) that the slope of the line so obtained represents the rate of growth of tubercle bacilli in whatever medium is being employed. This method has been used to great advantage to measure the rate of growth of tubercle bacilli under a variety of conditions (5, 6). The possibility exists that the present results obtained with mice may represent an equivalent situation.

Different numbers of tubercle bacilli are injected intravenously and a measurement is made (the median survival time) of the time required for them to multiply to a certain standard number in the mice. The latter statement is based on the assumption that, when the population of tubercle bacilli within a mouse has attained a certain number, death will result. This would appear to be a reasonable assumption since, regardless of the mechanism of death, there should be some close relationship between the number of tubercle bacilli producing an infection and inability of the mice to survive. If this is true and if the tubercle bacilli multiply at the same rate in the mice regardless of the size of the infecting dose, a relationship approximating linearity should be obtained when the data are plotted as demonstrated in figure 2. The slope of the line obtained would then represent the rate of multiplication of the tubercle bacilli in the mice.

Should the above hypothesis be correct, it would permit the acquisition of information regarding the activity of tubercle bacilli *in vivo* which has hitherto been impossible to obtain. For example, since the reciprocal of the slope of the line represented in figure 2 may be a measure of the rate of growth of tubercle bacilli *in vivo*, it would be possible to calculate from this the generation time (time for one cell division) *in vivo*.

$$\text{Generation time} = \frac{\text{Log } 2}{k}$$

where k equals the reciprocal of the slope of the line shown in figure 2.

When this was done with the data herein presented, it was found that the tubercle bacilli had a generation time of 4.6 days. This can be compared with the maximum rate of multiplication hitherto observed *in vitro* of approximately fourteen hours (4, 5, 6). It would be possible to conduct similar experiments and subject the mice to the action of chemotherapeutic agents, or to alterations in their physical environment or their state of immunity. Measurements could then be

made with some degree of accuracy of the effect of any one of these upon the rate of multiplication of tubercle bacilli *in vivo*. That this would be of inestimable value in quantitating such effects is easily recognizable.

Consideration of figure 2 shows also that there is an intercept on the abscissa which is of importance. If the reciprocal of the slope of the straight line is a measure of the rate of growth, then the intercept on the abscissa should represent the dose of tubercle bacilli resulting in immediate death of mice under the conditions of the experiments. This intercept, as calculated from the data, equals 7.8 mg. of tubercle bacilli. The fairly close agreement between this value and the direct experimental findings (table 1) tends to strengthen the hypothesis. Since this intercept should represent the dose of tubercle bacilli resulting in immediate death, experiments could be conducted in this manner to determine the effect of any administered agent or condition on the point of intercept. If the value of the intercept were increased, such a result could be interpreted to mean that the treatment had increased the resistance of the mice to this lethal dose of tubercle bacilli. If, on the other hand, the value were decreased, the converse would be true.

It follows, from what has been said, that data obtained from a single such experiment could be analyzed to determine the effect of any agent or condition on both the slope of the line, that is, the rate of growth, and upon the intercept, that is, the susceptibility of mice to a certain lethal dose of tubercle bacilli.

Other factors which might affect the above interpretation of the data should also be considered. For example, very little is known about the immediate fate of tubercle bacilli when injected into mice. For a variety of reasons, some of the bacteria may be killed or so affected that they are unable to grow. The number killed, or unable to multiply, may be constant or may be inversely or directly proportional to the number injected. How these factors, if operative, would affect the validity of the values for the slope and intercept will have to be determined by future experimentation. In any event, the significance of differences between experimentally determined values would not be affected.

The possibility also should be considered that there may be a lag period before *in vivo* multiplication begins even though significant numbers of tubercle bacilli are not killed following intravenous injection into mice. Because of the rapidity with which the animals infected with the large doses of tubercle bacilli succumb, this would not seem likely. In any event, even if there were an appreciable lag period, it would not affect the slope of the line but would tend only to increase the value for the intercept. The relatively close agreement between the value of the intercept in figure 2 and the experimentally determined minimum lethal dose of tubercle bacilli again makes this appear unlikely.

The state of natural resistance of the mouse to tuberculous infection is also important. The close approach to linearity found between the logarithm of the infecting dose and the median survival time would tend to indicate that this factor is acting uniformly regardless of the size of the infecting dose. On the other hand, the small deviations from linearity observed in figure 2 may be due to

the fact that the natural resistance of the mouse is operating more effectively against the smaller doses employed than against the larger.

The possibility also exists that tubercle bacilli do not multiply at the same rate in all of the organs of the mouse. There is evidence, particularly with smaller infecting doses (7), that the most rapid proliferation occurs in the lung. It is possible, and perhaps even probable, that death of mice infected with tubercle bacilli is primarily the result of pulmonary involvement. This would mean that the rate of growth, as measured by our data, is representative of the rate of multiplication of tubercle bacilli in the lung primarily rather than in other organs.

We would like to emphasize that we are dealing here with only a portion of the dosage, time of death, response curve. Those mice, as mentioned previously, which were injected with doses smaller than 0.5 mg. did not show as uniform a response in terms of time of death. Further work is being done on the response of mice to smaller infecting doses of tubercle bacilli and the data will be reported subsequently.

Worthy of mention is the immediate lethal effect of the large (10 mg. and 12 mg.) doses of tubercle bacilli. While the absence of such an effect with equivalent doses of heat-killed tubercle bacilli would suggest the absence of nonspecific vascular effects as a cause of death, it is not believed that this possibility has been completely eliminated. The possibility should not be overlooked, however, that some specific toxic factor is involved. Work is at present under way in an attempt to resolve this problem.

SUMMARY

Data are presented which indicate that, when mice are injected intravenously with large doses of living virulent human-type tubercle bacilli (H37Rv), a relationship approximating linearity is obtained when the logarithms of the infecting doses are plotted against their respective median survival times.

The implications of these findings are discussed and the possibility is suggested that the analysis of such data may furnish information relative to the rate of multiplication of tubercle bacilli in mice and the state of resistance or immunity of mice to tuberculous infection.

SUMARIO

La Relación entre el Tamaño de la Dosis Infectante de Bacilos Tuberculosos y el Tiempo de Sobrevivencia de los Ratones

Los datos presentados indican que, cuando se inyectan endovenosamente a los ratones grandes dosis de bacilos tuberculosos vivos virulentos de tipo humano (H37Rv), obtiénese una relación cuasilineal al trazar los logaritmos de las dosis infectantes contra sus respectivos tiempos medios de sobrevivencia.

Al discutir el alcance de dichos hallazgos, apúntase la posibilidad de que el análisis de esos datos facilite información relativa a la velocidad de la multi-

plicación de los bacilos tuberculosos en los ratones y al estado de resistencia o inmunidad de esos animales a la infección tuberculosa.

RESUME

Relation entre la valeur de la dose de bacilles tuberculeux inoculée à la souris et le temps de survie de l'animal

Présentation de données indiquant que, lorsqu'on inocule à des souris des bacilles humains virulents vivants (H37Rv) par voie intraveineuse on obtient une relation sensiblement rectiligne, quand on considère le logarithme des doses d'inoculation comparé au temps de survie moyen respectif.

Les implications de ces informations sont étudiées, l'hypothèse qu'une analyse de ces données pourrait éclairer le problème de la multiplication du bacille tuberculeux chez la souris et des conditions de résistance ou d'immunité à l'infection tuberculeuse chez cet animal est formulée.

REFERENCES

(1) Youmans, G. P., and Youmans, A. S.: The assessment of antituberculous chemotherapeutic activity in mice using virulent human-type tubercle bacilli, Am. Rev. Tuberc., 1951, *64*, 541.

(2) Youmans, G. P. and Raleigh, G. W.: The use of mice in experimental chemotherapy of tuberculosis: III. The histopathologic assay of chemotherapeutic action, J. Infect. Dis., 1948, *82*, 221.

(3) Litchfield, J. T., Jr.: A method for rapid graphic solution of time per cent effect curves, J. Pharmacol. & Exper. Therap., 1949, *97*, 399.

(4) Youmans, G. P., and Youmans, A. S.: A method for the determination of the rate of growth of tubercle bacilli by the use of small inocula, J. Bact., 1949, *58*, 247.

(5) Youmans, G. P., and Youmans, A. S.: The effect of bovine plasma fractions on the growth of *Mycobacterium tuberculosis* var. *hominis*, J. Bact., 1950, *60*, 561.

(6) Youmans, A. S., and Youmans, G. P.: The growth of recently isolated strains of *Mycobacterium tuberculosis* var. *hominis* in liquid media, J. Bact., 1950, *60*, 569.

(7) Raleigh, G. W., and Youmans, G. P.: The use of mice in the experimental chemotherapy of tuberculosis: II. Pathology and pathogenesis, J. Infect. Dis., 1948, *82*, 197.

THE ASSESSMENT OF ANTITUBERCULOUS CHEMOTHERAPEUTIC ACTIVITY IN MICE, USING VIRULENT HUMAN-TYPE TUBERCLE BACILLI[1, 2]

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INTRODUCTION

The rapid and accurate estimation of the degree of antituberculous chemotherapeutic activity *in vivo* of synthetic organic compounds or antibiotics is of considerable practical importance. In previous reports from this laboratory on the antituberculous activity of streptomycin (1), para-aminosalicylic acid (2), chloramphenicol (Chloromycetin) (3), and viomycin (4) on experimental tuberculous infections in mice, the activity of the drugs was determined primarily by making an estimation of the relative amount of gross and microscopic tuberculous involvement in the lungs of treated and control animals. While this method was rapid and permitted the detection of chemotherapeutic effect, it suffered from the subjective nature of the evaluation which made a measurement of the actual degree of chemotherapeutic action impossible.

Recognizing these limitations, McKee, Rake, Donovick, and Jambor (5), Donovick, McKee, Jambor, and Rake (6), and Rake, Jambor, McKee, Pansy, Wiselogle, and Donovick (7) developed a more standardized method for testing the antituberculous activity of compounds in mice. The basis for this test rested on the calculation of the median survival time of mice following intravenous infection with a standardized dose of the Ravenel strain of *Mycobacterium tuberculosis* var. *bovis*. This method permitted an objective and far more precise measurement of chemotherapeutic effect in mice.

Baker, Schlosser, and White (8) have reported the details of a similar method, but which differed from that of Rake and his associates in the strain of *M. tuberculosis* var. *bovis* employed as the infective agent, in the manner of calculation of the median survival time, and in the method of statistical analysis of the data.

Both of the above groups of workers employed a bovine variety of *M. tuberculosis* because they were unable to obtain a uniform response, in terms of mortality, in mice infected with the human variety of *M. tuberculosis*. Death did not occur in 100 per cent of the animals within a reasonable time (8) nor did the death of the infected animals always follow a normal frequency distribution curve (7). While the dosage of the human variety of *M. tuberculosis* employed by Baker, Schlosser, and White was not given, the maximum dose of the human variety of *M. tuberculosis* employed by Rake and his associates was

[1] From the Department of Bacteriology, Northwestern University Medical School, Chicago 11, Illinois.

[2] This work was aided, in part, by a research grant from Parke, Davis & Company, Detroit 32, Michigan.

0.1 mg. per mouse. Martin (9) had previously reported a method based on calculation of the average survival time of mice infected with large doses, 1.0 mg., of a human-type tubercle bacillus.

The advantages to be gained from employing an objective and more precise method for the estimation of chemotherapeutic activity of antituberculous drugs in mice are numerous. The present investigators have been reluctant to abandon the use of *Mycobacterium tuberculosis* var. *hominis* as a test organism, however, since human-type tubercle bacilli cause the vast majority of infections in the humans who will be treated with drugs selected on the basis of animal tests.

In view of the report of Martin (9) and the generally recognized fact that there is an inverse relationship between the number of tubercle bacilli injected and the survival time of experimental animals, an attempt has been made to develop a method similar to that employed by Rake and his associates (7) and Baker, Schlosser, and White (8), using larger infecting doses of *M. tuberculosis* var. *hominis*.

Since the impression may have been gained from the publications of Rake and his associates (7) and Baker, Schlosser, and White (8) that human-type tubercle bacilli cannot be employed for such standardized tests in mice, observations are presented in this report which indicate that *M. tuberculosis* var. *hominis* is just as suitable a test organism as the bovine variety, providing a sufficiently large infecting dose is employed.

METHODS

Mouse strain: Mice of the "Strong A" strain weighing between 18 and 22 gm. were employed in all experiments except where otherwise indicated.

Strain of tubercle bacillus: Because of its high degree of virulence, ease of maintenance of virulence, known characteristics, and widespread use as a test organism, the H37Rv strain of *Mycobacterium tuberculosis* var. *hominis* was employed. This organism was maintained in the laboratory under the same conditions, and suspensions for inoculation into mice were prepared and standardized in the same manner as we have reported previously in detail (10). The infecting dose of 1.0 mg., wet weight, was administered intravenously in a volume of 0.4 ml. of 0.01 M phosphate buffer. Detailed data on the response of mice to various doses of tubercle bacilli administered intravenously will be given in a subsequent publication.

Chemotherapeutic experiments: The chemotherapeutic experiments were conducted in a manner similar to that previously reported (10). Administration of streptomycin and viomycin was by subcutaneous injection and was begun on the day the mice were infected. Para-aminosalicylic acid (PAS) was administered by the drug diet method and was begun on the day before the mice were infected. Following infection all mice were observed daily and the time of death of each mouse was recorded. In order to obtain confirmation of the cause of death, each mouse was autopsied and an estimation made of the amount of gross tuberculosis present in the lungs.

Cumulative per cent mortality was plotted against time on logarithmic probability paper, and the median survival time (ST_{50}) and 95 per cent confidence limits were calculated by the method of Litchfield (11).

RESULTS

Mice of the "Strong A" strain when infected intravenously with 1.0 mg. of H37Rv strain of *M. tuberculosis* showed a strikingly uniform response. The mice

appeared relatively normal for the first week, but thereafter began to appear emaciated, and soon died. At autopsy, the lungs were large and edematous and showed the presence of many small, discrete "tubercles." To date, in 28 separate experiments, each of which employed 10 to 30 mice, a total of 480 untreated control mice have been infected in this manner.

In figure 1 may be seen the mortality distribution of 467 of these 480 mice. Eleven mice which died before the seventh day of causes other than tuberculosis, one mouse which survived 54 days, and one mouse which survived 150 days are not included. The corrected mortality from tuberculosis in this group of mice is 98.38 per cent. It can be seen from figure 1 that the death of these mice followed a distribution which approached that of a normal frequency distribution curve.

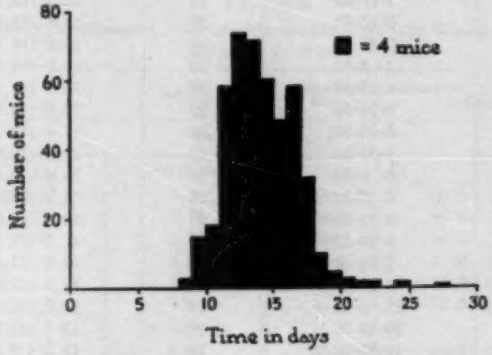

FIG. 1. Distribution of time of death of 467 untreated mice infected with 1.0 mg. of *M. tuberculosis* var. *hominis* (H37Rv).

The median survival time of each group may be seen in table 1. In the 28 experiments, covering a period of approximately a year, the median survival times of the groups of mice infected with tubercle bacilli varied between 11.5 and 15.8 days, indicating a high degree of reproducibility for this type of test.

The effect of sex and weight on the survival of mice infected intravenously with 1.0 mg. of H37Rv is given in table 2. It is apparent that the sex of the animals exerted no influence on their response to tuberculous infection. Moreover, within the limits tested, the weight of the animals did not markedly affect their survival. There was no statistically significant difference between the response of mice averaging 29.8 gm. in weight and those averaging 24.9 gm., or between those averaging 24.9 gm. and those averaging 19.9 gm., or between those averaging 19.9 gm. and those averaging 15.1 gm. There was, however, a statistically significant difference between the median survival times of the 29.8 gm. group and the 19.9 gm. group, between those averaging 24.9 gm. and those averaging 15.1 gm., and, as would be anticipated, between those averaging 15.1 gm. and those averaging 29.8 gm. The weights given in table 2 are averages, but

in no case did the weights of the individual mice vary more than a plus or minus 2 gm. from the average. Since weight and age are to a certain extent interdependent, the results of this experiment may also reflect the influence of age on the response of this strain of mice to tuberculous infection.

TABLE 1

Median Survival Times of 28 Groups of Mice Infected Intravenously with 1.0 Mg. H37Rv

EXPERIMENT NUMBER	DATE MICE INFECTED	NUMBER OF MICE	MEDIAN SURVIVAL TIME (95 PER CENT CONFIDENCE LIMITS)
1	2-24-50	30	13.7 (13.0–14.3)
2	3- 3-50	20	13.7 (12.6–14.9)
3	3-10-50	20	14.5 (13.7–15.4)
4	3-17-50	20	13.5 (12.7–14.3)
5	3-17-50	20	14.0 (12.8–15.3)
6	3-31-50	20	15.8 (14.5–17.2)
7	4- 7-50	30	15.0 (14.4–15.7)
8	4-15-50	30	15.0 (14.4–15.5)
9	5-16-50	20	15.0 (13.9–16.1)
10	5-19-50	20	14.0 (13.2–14.9)
11	5-26-50	10	14.0 (13.0–15.0)
12	6- 9-50	10	14.0 (13.1–14.9)
13	6-13-50	20	14.1 (13.5–14.5)
14	6-21-50	20	13.5 (13.0–14.0)
15	6-30-50	20	12.5 (12.0–13.0)
16	7-14-50	10	13.0 (11.6–14.5)
17	7-21-50	20	13.5 (13.0–14.0)
18	10- 5-50	40	13.0 (12.9–14.1)
19	10-13-50	10	12.5 (11.1–14.1)
20	10-27-50	10	11.5 (9.5–13.9)
21	11-17-50	10	13.0 (11.1–15.2)
22	12- 4-50	10	13.0 (12.5–14.6)
23	12- 8-50	10	11.5 (10.5–12.6)
24	1- 3-51	10	12.5 (11.3–13.9)
25	1- 4-51	10	13.8 (13.0–14.6)
26	1-19-51	10	14.0 (12.6–15.5)
27	1-26-51	10	14.0 (13.1–14.9)
28	2- 8-51	10	12.0 (10.3–14.0)
Total.....		480	

The effect on the median survival time of mice infected as described above was tested by using the known chemotherapeutic agents, PAS, streptomycin, and viomycin, in varying doses as indicated in table 3. It is apparent that treatment with these drugs increases the median survival time. Furthermore, there is a direct relationship between the amount of drug administered and the median survival time. The reproducibility of such chemotherapeutic experiments is indicated by the data given in table 4. This table shows the results of using 150 mice in a total of 13 separate experiments when treated with PAS. The median

TABLE 2

Effect of Sex and Weight on Survival of Mice Infected Intravenously with 1.0 Mg. H37Rv

SEX OR AVERAGE WEIGHT OF MICE	NUMBER OF MICE	MEDIAN SURVIVAL TIME (95 PER CENT CONFIDENCE LIMITS)
Male	62	13.5 (12.9–14.0)
Female	62	13.0 (12.5–13.5)
29.8 gm.	30	16.5 (15.9–17.0)
24.9 gm.	30	16.2 (15.2–17.3)
19.9 gm.	30	15.0 (14.5–15.5)
15.1 gm.	30	14.0 (13.1–14.9)

TABLE 3

Relative Activity of Streptomycin, Para-Aminosalicylic Acid, and Viomycin on Tuberculous Infection in Mice

DRUG	DOSAGE	NUMBER OF MICE	MEDIAN SURVIVAL TIME (95 PER CENT CONFIDENCE LIMITS)
Treated 14 Days			
	mg. per day		
Streptomycin*	1.5	40	60.0 (53.1–67.8)
	0.75	40	45.0 (39.1–51.7)
	0.375	40	21.0 (19.2–22.8)
	0.187	39	21.0 (19.0–23.1)
	0.093	40	17.5 (16.5–18.5)
	0.0	39	12.5 (11.5–13.2)
	per cent in diet		
PAS	1.0	40	44.0 (41.9–50.6)
	0.5	38	29.0 (26.6–32.7)
	0.25	35	25.0 (22.1–28.2)
	0.125	35	21.5 (20.4–22.5)
	0.062	39	15.5 (14.3–16.7)
	0.0	38	13.5 (12.6–14.3)
	mg. per day		
Viomycin	3.0	20	60.0 (50 –71)
	1.5	20	21.5 (18.9–24.4)
	0.75	20	16.8 (15.9–17.6)
	0.375	20	15.8 (14.6–17.0)
	0.187	20	15.0 (14.0–16.0)
	0.0	20	12.5 (12.0–13.0)
Treated 28 Days			
	mg. per day		
Viomycin	12.0	10	120.0 (103 –135)
	6.0	10	110.0 (86 –140)
	3.0	10	83.0 (70 –98)
	0.0	10	15.8 (14.5–17.2)

* Streptomycin sulfate.

survival times varied in the different experiments between 21.5 and 30 days. The over-all median survival time of all the mice given in table 4 was 26.0 days (24.3 to 27.8). Fourteen of the 150 mice died early, that is, before the seventh day, of causes other than tuberculosis, leaving 136 mice (90.6 per cent) which died of tuberculosis. Of the mice which died of tuberculosis, all died between the nineteenth and fifty-eighth days, and 115 (84.5 per cent) died between 15 and 35 days.

TABLE 4

Median Survival Times of 13 Groups of Mice Infected with 1.0 Mg. H37Rv and Treated by Incorporating 0.5 Per Cent PAS in Diet

EXPERIMENT NUMBER	DATE MICE INJECTED	NUMBER OF MICE	MEDIAN SURVIVAL TIME (95 PER CENT CONFIDENCE LIMITS)
1	6- 9-50	10	23.5 (22.1-24.9)
2	6-21-50	20	28.5 (25.2-32.3)
3	7-14-50	10	21.5 (16.6-27.2)
4	7-21-50	10	28.0 (20.0-30.2)
5	10- 5-50	20	30.0 (26.8-33.6)
6	10-13-50	10	23.5 (21.6-25.6)
7	10-27-50	10	22.5 (20.8-24.3)
8	11- 3-50	10	25.0 (23.8-26.3)
9	11-17-50	10	25.5 (23.9-27.2)
10	12- 4-50	10	27.0 (22.5-32.4)
11	12- 8-50	10	27.5 (23.9-31.6)
12	1- 3-51	10	24.0 (21.6-26.6)
13	1-19-51	10	24.0 (21.6-26.6)

DISCUSSION

The results given herein show that the "Strong A" strain of mice, if given intravenously a large (1.0 mg.) infecting dose of the H37Rv strain of *M. tuberculosis*, will die rapidly and in a relatively uniform manner. This permits the calculation of the median survival time and the estimation of the chemotherapeutic activity of drugs on the basis of an increase in the median survival time of the animals being treated. This method is based upon, and is similar to, the methods described by Baker, Schlosser, and White (8) and Rake and his associates (7). It differs from the methods described by the above investigators in that human-type tubercle bacilli were employed instead of bovine type, in the strain of mice employed, and in the size of the infecting dose of tubercle bacilli. The above writers reported that human-type tubercle bacilli were unsuitable for this kind of test, but we believe their findings may have been due to the fact that insufficiently large infecting doses of human-type tubercle bacilli were employed. The advantages of employing a more rapid and more accurate test for the chemotherapeutic activity of antituberculous agents has been pointed out by Baker, Schlosser, and White and by Rake and his associates. The test described in the present report has largely been substituted in this laboratory for the one previously employed in which the chemotherapeutic activity of antituberculous drugs was determined by estimating the relative amount of gross

and microscopic tuberculosis in treated animals as compared with untreated animals.

It is of interest to compare the response of mice infected with large doses of human-type tubercle bacilli, as found in the present study, with that of mice infected with bovine-type tubercle bacilli as obtained under the conditions set up by Rake and his associates (7) and Baker, Schlosser, and White (8) following treatment with known chemotherapeutic agents, such as streptomycin and PAS.

TABLE 5

Comparison of Median Survival Times (ST_{50}) of Mice Treated with PAS

DRUG INTAKE		MEDIAN SURVIVAL TIME IN DAYS		INCREASE IN MEDIAN SURVIVAL TIME BY PAS	
Mg. of PAS per Kg. per Day					
Baker, Schlosser, and White	Youmans and Youmans	Baker, Schlosser, and White	Youmans and Youmans	Baker, Schlosser, and White	Youmans and Youmans
2,800	1,900	21.0	44.0	7.0	30.5
1,200	1,000	17.0	29.5	3.0	16.0
550	530	15.0	25.0	1.0	11.5
270	234	15.0	21.5	1.0	8.0
0	0	14.0	13.5	0.0	0.0

TABLE 6

Comparison of Median Survival Times (ST_{50}) of Mice Treated with PAS

DRUG INTAKE		MEDIAN SURVIVAL TIME IN DAYS		INCREASE IN MEDIAN SURVIVAL TIME BY PAS	
M-moles of PAS per Kg. per Day					
Rake *et al.*	Youmans and Youmans	Rake *et al.*	Youmans and Youmans	Rake *et al.*	Youmans and Youmans
12.77	12.40	28.11	44.0	8.25	30.5
10.08		26.81		6.95	
6.54	6.53	25.65	29.5	5.79	16.0
5.06		24.06		4.20	
3.51	3.46	20.06	25.0	1.02	11.5
2.77		22.92		3.02	
1.67	1.53	22.0	21.5	2.14	8.0
0	0	19.86	13.5	0.0	0.0

Tables 5 and 6 show our results for mice treated with PAS as compared with those of Rake and associates and Baker, Schlosser, and White. The actual drug intake in our experiments was calculated and is given in the tables in the same terms as those used by the other authors. The increase in median survival time was calculated by subtracting the median survival time of the control groups of animals from the survival times of the treated groups of animals. Since Rake and his associates did not give the median survival time of the control groups for individual experiments, the median survival time given by these authors has been used as representative of the response of untreated animals.

It is apparent that under the conditions of the present study the mice survived for much longer periods following treatment with PAS than did the mice used by either of the other two groups of investigators. In the experiments of Baker, Schlosser, and White, the mice were administered PAS for fourteen days, the same treatment period we employed. The median survival time of their control animals was almost the same as that obtained with our control group,

TABLE 7

Comparison of Median Survival Times (ST_{50}) of Mice Treated with Streptomycin

DRUG INTAKE		MEDIAN SURVIVAL TIME IN DAYS		INCREASE IN MEDIAN SURVIVAL TIME BY STREPTOMYCIN	
Mg. of Streptomycin per Kg. per Day					
Baker, Schlosser, and White	Youmans and Youmans*	Baker, Schlosser, and White	Youmans and Youmans	Baker, Schlosser, and White	Youmans and Youmans
200		42.0		30.0	
100	75.0	30.0	60.0	28.0	47.5
50	37.5	23.0	45.0	11.0	22.5
25	18.75	15.0	21.0	3.0	8.5
0	9.37		21.0		8.5
0	4.68		17.5		5.0
0	0	12.0	12.5	0.0	0.0

* Streptomycin sulfate.

TABLE 8

Comparison of Median Survival Times (ST_{50}) of Mice Treated with Streptomycin

DRUG INTAKE		MEDIAN SURVIVAL TIME IN DAYS		INCREASE IN MEDIAN SURVIVAL TIME BY STREPTOMYCIN	
M-moles of Streptomycin per Kg. per Day					
Rake *et al.*	Youmans and Youmans*	Rake *et al.*	Youmans and Youmans	Rake *et al.*	Youmans and Youmans
	0.10		60.0		47.5
0.039	0.05	32.5	45.0	12.64	32.5
0.026	0.025	30.2	21.0	10.34	8.5
0.0173	0.0125	27.5	21.0	7.64	8.5
0.0091	0.00625	26.8	17.5	6.94	5.0
0.0	0.0	19.86	12.5	0.0	0.0

* Streptomycin sulfate.

suggesting that the severity of the tuberculous infection was similar in both cases. Even so, PAS showed, under the conditions of the present experiments, a much greater degree of therapeutic activity. In the experiments reported by Rake and his associates, treatment was continued for twenty-one days as opposed to the fourteen days of the present study; yet, even though the average daily drug intake was very similar in the two sets of experiments, PAS showed a much greater therapeutic activity under the conditions of the present study.

Tables 7 and 8 show similar data for streptomycin. In no case in the present experiments was exactly the same drug dosage used as in the experiments of

either of the other groups of investigators. Nevertheless, a considerably greater increase in the median survival time was noted even with smaller doses of streptomycin than those reported by Baker, Schlosser, and White. Our values for the increase in median survival time of mice treated with streptomycin are closer to those of Rake and his associates. It should be kept in mind, however, that the latter investigators treated their mice for three weeks, one week longer than the treatment period used in the present study. It appears from these comparisons that the test employed in the present investigation is, in general, considerably more sensitive than the tests previously reported.

Because of the differences in experimental conditions, it is impossible to specify the reasons for these considerable differences in chemotherapeutic effect. In view of the fact that human-type tubercle bacilli cause the vast majority of infections in human beings, however, it is believed that the possibility should be considered that the employment of human-type tubercle bacilli in such chemotherapeutic tests may give results which are more apt to be a reflection of the potential effectiveness of a drug for the treatment of the human disease than is true when bovine strains are used.

SUMMARY

A standardized test for antituberculous chemotherapeutic activity in mice is described which employs the virulent human-type tubercle bacillus strain H37Rv. When 1.0 mg. wet weight of this strain is administered intravenously into mice, the mortality is 100 per cent and the times of death appear to follow a normal frequency distribution curve. This permits the calculation of the median survival time which, under the conditions of the test, is approximately fourteen days. The chemotherapeutic agents streptomycin, para-aminosalicylic acid, and viomycin prolong the survival of mice infected in this manner to a degree which is proportional to the dose administered. The method appears to be considerably more sensitive than similar methods which employ virulent bovine-type tubercle bacilli as the infecting microorganisms.

SUMARIO

La Valoración de la Actividad Quimioterapéutica Antituberculosa en los Ratones Usando Bacilos Tuberculosos Virulentos de Tipo Humano

La prueba normalizada descrita para determinar la actividad quimioterapéutica antituberculosa en los ratones utiliza la cepa H37Rv virulenta del bacilo tuberculoso de tipo humano. Al inyectar endovenosamente 1.0 mg. de peso húmedo de esta cepa en los ratones, la mortalidad es de 100 por ciento y los tiempos de muerte parecen seguir una curva normal de distribución de la frecuencia. Esto permite calcular el tiempo medio de sobrevivencia que, en las condiciones de la prueba, es aproximadamente de catorce días. Los agentes quimioterapéuticos, estreptomicina, ácido para-aminosalicílico y viomicina, prolongan la sobrevivencia de los ratones infectados de esa manera, a un punto proporcional a la dosis administrada. Esta técnica parece ser considerablemente más delicada que las técnicas que emplean como microbios infectantes bacilos tuberculosos de tipo bovino.

RESUME

Détermination de l'action chimiothérapeutique antituberculeuse chez la souris, à l'aide de bacilles tuberculeux humains virulents

Description d'un test standard pour la détermination de l'action chimiothérapeutique dans la tuberculose de la souris par inoculation de bacilles tuberculeux humains virulents, souche H37Rv. Chez la souris l'inoculation de 1 mg. (poids mouillé) de cette souche, par la voie intraveineuse, détermine un taux de mortalité de 100% et l'animal succombe dans un délai conforme à la courbe de fréquence normale. Ceci permet de calculer le temps de survie moyen qui, dans les conditions de l'expérimentation, est approximativement de quatorze jours. Chez l'animal ainsi inoculé les agents chimiothérapeutiques, streptomycine, acide para-aminosalicylique, viomycine, le temps de survie est proportionnel à la dose administrée. La sensibilité de cette méthode est considérablement plus grande que celle des méthodes qui utilisent les bacilles bovins virulents comme agent d'inoculation.

REFERENCES

(1) Youmans, G. P., and McCarter, J. C.: Streptomycin in experimental tuberculosis: Its effect on tuberculous infections in mice produced by *M. tuberculosis* var. *hominis*, Am. Rev. Tubere., 1945, *52*, 432.

(2) Youmans, G. P., Raleigh, G. W., and Youmans, A. S.: The tuberculostatic action of para-aminosalicylic acid, J. Bact., 1947, *54*, 409.

(3) Youmans, G. P., Youmans, A. S., and Osborne, R. R.: The tuberculostatic action of Chloromycetin *in vitro* and *in vivo*, Proc. Soc. Exper. Biol. & Med., 1948, *67*, 426.

(4) Youmans, G. P., and Youmans, A. S.: The effect of viomycin *in vitro* and *in vivo* on *Mycobacterium tuberculosis*, Am. Rev. Tubere., 1951, *63*, 25.

(5) McKee, C. M., Rake, G., Donovick, R., and Jambor, W. P.: The use of the mouse in a standardized test for antituberculous activity of compounds of natural or synthetic origin: I. Choice and standardization of culture, Am. Rev. Tuberc., 1949, *60*, 90.

(6) Donovick, R., McKee, C. M., Jambor, W. P., and Rake, G.: The use of the mouse in a standardized test for antituberculous activity of compounds of natural or synthetic origin: II. Choice of mouse strain, Am. Rev. Tuberc., 1949, *60*, 109.

(7) Rake, G., Jambor, W. P., McKee, C. M., Pansy, F., Wiselogle, F. Y., and Donovick, R.: The use of the mouse in a standardized test for antituberculous activity of compounds of natural or synthetic origin: III. The standardized test, Am. Rev. Tuberc., 1949, *60*, 121.

(8) Baker, M. J., Schlosser, M. E., and White, H. J.: A method for evaluating antitubercular activity in mice, Ann. New York Acad. Sc., 1949, *52*, 678.

(9) Martin, A. R.: The use of mice in the examination of drugs for chemotherapeutic activity against *Mycobacterium tuberculosis*, J. Path. & Bact., 1946, *58*, 580.

(10) Youmans, G. P., and Raleigh, G. W.: The use of mice in experimental chemotherapy of tuberculosis: III. The histopathologic assay of chemotherapeutic action, J. Infect. Dis., 1948, *82*, 221.

(11) Litchfield, J. T., Jr.: A method for rapid graphic solution of time-per cent effect curves, J. Pharmacol. & Exper. Therap., 1949, *97*, 399.

BLOOD MEDIA FOR CULTURING TUBERCLE BACILLI[1,2]

Evaluation by Means of a Serial Dilution Method

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INTRODUCTION

In a previous paper (8) the observations of Koch and others concerning the value of blood for the cultivation of *Mycobacterium tuberculosis* were confirmed. It was demonstrated that human bank blood incorporated into a variety of agar bases was capable of initiating growth of as small an inoculum as 30 to 300 tubercle bacilli. This observation suggested that blood media should be examined for their diagnostic potential in tuberculous infections. The following report is concerned with such an investigation of four media using the method of diluting sputum concentrates to the limit at which growth of organisms can be obtained. This technique was introduced in the original study and is especially suited for cultural evaluation experiments.

MATERIALS AND METHODS

Corper-Cohn medium was made as directed (2) except that sterilization was accomplished by autoclaving in a steam-air mixture at 16 pounds pressure for 90 minutes. Löwenstein medium as modified by Jensen and Holm (4) was prepared as directed. The slants were solidified and sterilized by inspissation at 85 to 88°C. for 40 minutes.

The blood agar was prepared by hydrating the stated amount of the Difco basal medium in 1 per cent USP glycerin and sterilizing for 15 minutes at 121°C. The liquid base was gradually cooled in a water bath to 55°C. and bank blood containing standard acid citrate and dextrose solution was then added to a final concentration of 25 per cent. The medium was tubed, using sterile precautions, slanted, and incubated 48 hours at 37°C. to test for sterility. The agar-agar medium was prepared by dissolving 2 gm. of agar (Baltimore Biological Laboratory) in 100 ml. of distilled water and then sterilizing for 15 minutes at 121°C. The bank blood was added in the manner described above. Screw-cap test tubes measuring 5 by ¾ inches and containing 4 ml. of medium were used throughout the experiments.

Sputum specimens consisted of 24-hour samples collected from known tuberculous persons, most of whom had been or were receiving streptomycin, PAS, or both. The specimens were concentrated, using the trisodium phosphate technique (9). Tenfold serial dilutions, using single pipettes for each dilution, were prepared in tubes containing 0.85 per cent sodium chloride. The contents of each tube were thoroughly mixed prior to dilution and again at the time of inoculation of the media. Each medium received an inoculum of 0.1 ml. with the same pipette beginning with the lowest concentration and progressing to the highest. The order of inoculation of the four media was varied at random. The tubes were incubated at 37°C. in a slanted position for five days to permit good distribution of the inoculum and were then placed upright for the remainder of the period of observation. The first evidence of visible growth was recorded and verified by subsequent development of typical colonies. At the end of eight weeks the experiment was concluded, and stained preparations of atypical or suspicious colonies were examined microscopically. The pathogenicity of the strains for guinea pigs was not determined.

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[1] From the Department of Bacteriology, University of Oregon Medical School, Portland, Oregon.

[2] Aided by the Truman Collins Fund.

RESULTS

Four types of colonies have been described on human blood media (8). On agar-agar containing 25 per cent blood, the characteristic colony is at first pinpoint in size and gradually increases to approximately 8 mm. in diameter. Mature colonies are discrete, irregular, dry, rough, slightly elevated, and exhibit a small nipple-like center. With age the grayish surface often assumes a reddish cast. Growth is visible approximately one week earlier on blood than on either the Corper-Cohn or the Lōwenstein medium. This observation has been made previously (8) and is attributed, in part, to the marked color contrast between the

TABLE 1

Cultures of Tubercle Bacilli Obtained by Serial Dilution of Concentrated Sputums on Various Media

GROUP	MEDIA	SPUTUM DILUTIONS							
		10^{-0}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	Total
		(12)*	(19)						
I	Corper-Cohn	0	0	0	0	0	0	0	0
	Lōwenstein	2	6	1	0	0	0	0	9
	BAB + 1 per cent G + 25 per cent blood†	6	10	5	1	0	0	0	22
	AA + 25 per cent blood‡	9	12	5	1	0	0	0	27
		(12)	(29)						
II	Corper-Cohn	11	26	17	10	2	0	0	66
	Lōwenstein	12	28	27	18	6	1	1	93
	BAB + 1 per cent G + 25 per cent blood	9	24	22	14	4	1	1	75
	AA + 25 per cent blood	11	27	26	14	8	1	0	87
		(24)	(48)						
Total	Corper-Cohn	11	26	17	10	2	0	0	66
	Lōwenstein	14	34	28	18	6	1	1	102
	BAB + 1 per cent G + 25 per cent blood	15	34	27	15	4	1	1	97
	AA + 25 per cent blood	20	39	31	15	8	1	0	114

* Numbers indicate total sputums cultured in the dilution specified. All specimens were tested in dilutions of 10^{-1} or greater.

† Blood agar base plus 1 per cent glycerin plus 25 per cent citrated human bank blood.

‡ Agar-agar plus 25 per cent citrated human bank blood.

red background and the gray colonies and also to the excellent nutritional qualities of blood.

A total of 48 sputum concentrates were titrated by tenfold serial dilutions. Of these, 24 were inoculated beginning with undiluted (10^{-0}) material and the remaining 24 with a 10^{-1} dilution of the original concentrate. In Group I are included those sputums from which tubercle bacilli were not recovered in any dilution on one or more of the culture media. It can be seen that, of 126 trials, growth was obtained 9 times on Lōwenstein, 22 times on blood agar, 27 times on agar-agar, and not at all with the Corper-Cohn medium. The majority of

concentrates fell into Group II which is categorized by the fact that growth was evident in one or more dilutions with all four media. The Löwenstein medium appeared superior in this series and accounted for a total of 93 successful isolations out of a total of 186 trials.

Since all the media received an equal number of tests with each concentrated specimen of sputum, the cumulative scores are comparable. A total of 312 attempts at isolations were made with each medium. In the order of listing in the table the scores ranged from a low of 66 for the Corper-Cohn to a high of 114 for the agar-agar medium containing bank blood. The chi-square test indicates that the last three media in the table are comparable among themselves and equal to each other in performance. On the other hand, the cultural data with the same three media either combined or taken separately are significantly better than those using the Corper-Cohn medium alone ($P = <0.005$). The results indicate that the blood media are at least as good for diagnostic purposes as the Löwenstein medium. This fact is of particular interest because of recent evaluation studies in which the Löwenstein medium was selected over the Petragnani, American Trudeau Society, and Dubos oleic acid-albumin-agar media (5, 6, 7).

DISCUSSION

Most reports dealing with diagnostic media are based on a series of simultaneous cultures of tubercle bacilli from concentrates of which a certain percentage yield growth (5, 6). The serial dilution method described herein should be preferred for such evaluation studies for the following reasons. Organisms are isolated under diagnostic conditions, and the use of laboratory strains is avoided. Since the inoculum is graded by serial dilution, studies can be limited to those specimens which are known to contain tubercle bacilli. The graded inoculum also makes possible a separation of specimens into those which contain large and those which contain small numbers of viable organisms. This advantage is desirable because the number of organisms introduced into the culture medium is an important limiting factor for growth (1, 2, 8). Statistically valid data can be obtained with relatively few cultures, provided each step in the technique is carefully standardized. It would also be possible to determine from preliminary experiments just how extensive a series would be required to substantiate any observed difference. On the other hand, the method is subject to the criticisms of all serial dilution techniques, namely, unequal distribution of organisms and variability of endpoints. The results which have been obtained using the procedures herein described have proved sufficiently reliable, as judged by comparison with other planting techniques (8) and by observations relative to the rate and constancy of growth in tubes containing serial inoculations, to obviate the danger of significant error. Refinements in procedure, such as grinding of sediments with wetting agents, might possibly be utilized but as yet have not been attempted.

The failure of the Corper-Cohn medium to equal the performance of the others may not have been due to its lack of growth-promoting qualities for tubercle bacilli but rather to the method of sterilization used which involved

heating in an air-steam mixture at 16 pounds pressure for 90 minutes. The choice of this procedure was based on a statement by Corper and Cohn (2) that autoclaving for 15 minutes was satisfactory. Previous experience with the Corper-Cohn medium prepared in this laboratory had indicated that it would grow small numbers of organisms (8). A definitive statement concerning the final performance of the Corper-Cohn medium will necessitate further studies involving the effects produced by various methods of sterilization.

The problem of contamination is an important one in routine diagnostic work, and it might be assumed that the blood media would be more likely to support the growth of extraneous organisms and fungi than the others which contain malachite green. Actually the incidence of contamination was not significantly different for each of the media and did not exceed 6 tubes of a total 312 despite the extensive manipulation for dilutions and inoculations. If proper precautions are taken during the concentration technique (9) and during transfer of sediments and supernates, contamination is not likely to be a serious difficulty with blood media.

As has already been pointed out (8), a number of investigators have utilized blood alone or in combination with various bases as a menstruum for the cultivation of *M. tuberculosis*. In addition, the studies of Corper and Damerow (3) call attention to the fact that defibrinated and inspissated dog-, rabbit-, guinea pig-, and hen-blood were capable of growing as small an inoculum of tubercle bacilli as the glycerin–egg-yolk medium. The incorporation of blood into an agar base serves to eliminate the possible deleterious effect of heat (8) and avoids the use of a liquid medium in routine diagnostic work. Although agar-agar has been described as inhibitory for tubercle bacilli (1), the agar medium which contained a final concentration of 1.6 per cent was as efficient as any of the others used. In the presence of 25 per cent bank blood, no evidence of inhibition by agar-agar was obtained. Furthermore, the inclusion of this polysaccharide has served to stimulate interest in the growth-enhancing qualities of various components of blood for tubercle bacilli. The results of such experiments, which will be reported separately, indicate that washed or lysed erythrocytes, plasma, and serum are capable of initiating growth of small inocula. Studies of plasma fractions and erythrocyte products are in progress.

It will be noted from the table that several of the strains of tubercle bacilli selected a particular medium for initiating growth. Striking examples may be seen in the Group I cultures in which preference for blood was dominant. Other organisms grew best on egg-yolk media. This discrepancy merely serves to stress the well-known fact that variability is an outstanding characteristic of *M. tuberculosis* and that a maximum efficiency for isolation techniques is not likely to be achieved by the use of any single medium. In our hands, the Löwenstein and the blood media proved equally sensitive. It is believed, therefore, that blood media should be included, together with others, in routine diagnostic cultures of the type used in larger institutions. It should be the medium of choice for small laboratories and hospitals in which the practice of culture for tuberculosis is limited by the difficulties in preparing and procuring other types of media. The data also indicate that such ingredients as beef heart infusion, tryptose, and

glycerin are not essential for the growth of tubercle bacilli in the presence of adequate quantities of blood. Additional experiments will be required, however, to elucidate the role of glycerin; and, for routine diagnostic work, the medium containing blood agar base, 1 per cent glycerin, and 25 per cent bank blood is recommended. Refrigerated tubes of this medium have proved exceedingly stable in tightly sealed screw-cap tubes for as long as four months.

SUMMARY

A method, herein presented, of evaluating culture media for their ability to grow tubercle bacilli from serially diluted sputum concentrates possesses the following advantages: (*1*) Organisms are isolated under routine diagnostic conditions; (*2*) a minimal inoculum is assured at some point in the titration; (*3*) specimens which do not contain tubercle bacilli can be safely excluded; (*4*) the data are comparable, and statistically valid results can be obtained with relatively few cultures.

Media containing human blood were shown to be equal to the Löwenstein medium by the serial dilution method. In fact, agar-agar alone incorporated with 25 per cent bank blood scored highest, with 114 positive results of a total of 312 trials. It is believed, therefore, that blood-containing media will prove preferable to the others because they are economical, readily available, easily prepared, stable on storage, and grow tubercle bacilli rapidly from small inocula.

SUMARIO

Medios Sanguíneos para el Cultivo de Bacilos Tuberculosos: Valuación por Medio de una Técnica de Dilución Seriada

La técnica descrita para valuar medios de cultivo conforme a su capacidad para formar colonias de bacilos tuberculosos de diluciones seriadas de esputo concentrado posee las siguientes ventajas: (1) los microbios quedan aislados en condiciones corrientes de diagnóstico; (2) hay seguridad de un inóculo mínimo en algún punto de la titulación; (3) permite excluir positivamente los ejemplares que no contienen bacilos tuberculosos; (4) los datos son comparables, y pueden obtenerse resultados estadísticamente válidos con relativamente pocos cultivos.

Los medios que contenían sangre humana resultaron ser iguales al medio de Löwenstein con la técnica de dilución seriada. En efecto, el agar-agar solo, unido a 25 por ciento de sangre del banco, alcanzó la marca más alta, con 114 resultados positivos en un total de 312 pruebas. Parece, pues, que los medios sanguíneos resultarán preferibles a los demás por ser económicos, accesibles, fáciles de preparar, estables para conservación y formar rápidamente de pequeños inóculos colonias de bacilos tuberculosos.

RESUME

Milieux au sang pour la culture du bacille tuberculeux: Leur évaluation par la méthode des dilutions en série

La méthode des dilutions en série présentée pour l'évaluation des milieux destinés à la culture des bacilles tuberculeux à partir de spécimens de crachats

concentrés possède les avantages suivants: (1) les bacilles sont isolés dans les conditions habituelles du diagnostic; (2) un inoculum minime est obtenu en un point du titrage; (3) les spécimens qui ne contiennent pas de bacilles tuberculeux peuvent être exclus; (4) les données sont comparables et des résultats statitiques valides peuvent être obtenus avec un nombre de cultures relativement faible.

Par la méthode des dilutions en série, les milieux contenant du sang humain se sont montrés de valeur égale à celle du milieu de Löwenstein. En fait la valeur la plus grande a été reconnue à l'agar-agar incorporé à 25% de sang conservé qui a donné 114 résultats positifs sur 312 essais. En conséquence, on pense que les milieux au sang se montreront supérieurs aux autres parce qu'ils sont économiques, rapidement accessibles, facilement préparés, stables, donc faciles à conserver et qu'ils permettent d'obtenir rapidement des cultures à partir d'un inoculum minime.

Acknowledgment

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REFERENCES

(1) Corper, H. J., and Cohn, M. L.: The nutrient qualities of eggs for growing tubercle bacilli, Am. J. Hyg., 1933, *18*, 1.

(2) Corper, H. J., and Cohn, M. L.: Media for tubercle bacilli: An evaluation of different media for diagnostic cultures of tubercle bacilli, Am. Rev. Tuberc., 1942, *46*, 500.

(3) Corper, H. J., and Damerow, A. P.: The question of tubercle bacilli in the blood in advanced pulmonary tuberculosis, Am. Rev. Tuberc., 1933, *28*, 118.

(4) Holm, J., and Lester, V.: Diagnostic demonstration of tubercle bacilli, Acta tuberc. Scandinav., 1941, *16*, 3; Pub. Health Rep., 1947, *62*, 847.

(5) Melvin, I., Klein, G. C., Jones, W., and Cummings, M. M.: An evaluation of media for the diagnostic cultures of tubercle bacilli, Am. Rev. Tuberc., 1951, *63*, 459.

(6) Smith, C. R.: Clinical comparison of several culture media in the diagnostic demonstration of tubercle bacilli, Am. Rev. Tuberc., 1951, *63*, 470.

(7) Smith, C. R., and Cannon, M.: Report to the Committee on Medical Research and Therapy of the American Trudeau Society, 1948.

(8) Tarshis, M. S., and Frisch, A. W.: Blood media for the cultivation of *Mycobacterium tuberculosis*, Am. J. Clin. Path., 1951, *21*, 101.

(9) Tarshis, M. S., and Lewis, W. G.: Use of clorox and trisodium phosphate in demonstration of acid-fast bacilli in sputum, Am. J. Clin. Path., 1949, *19*, 688.

THE SUBCUTANEOUS ADMINISTRATION OF THE SODIUM SALT OF PARA-AMINOSALICYLIC ACID IN THE TREATMENT OF TUBERCULOSIS[1, 2]

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INTRODUCTION

Para-aminosalicylic acid (PAS) has become a widely used therapeutic agent in the treatment of clinical tuberculosis (1, 2). It is employed most frequently in combination with streptomycin to delay the emergence of a bacterial population predominantly resistant to streptomycin (3, 4) and less frequently in the treatment of patients for whom streptomycin is not currently indicated.

Serious reactions to PAS are rarely encountered (5) but many patients suffer uncomfortable gastrointestinal irritation, and not infrequently these persons are unable to ingest a sufficient amount of the drug to accomplish a therapeutic objective. Patients who have recently been subjected to surgical operation, or who are violently ill, unconscious, or for other reasons are unable to eat, may require parenteral administration of PAS and are likely to be those in greatest need of intensive and prolonged treatment. The writers believe that the parenteral administration of this drug may be truly life saving in certain critical types of tuberculous infection, especially following radical surgery and in cases of tuberculous meningitis and miliary tuberculosis.

The preferred method of parenteral administration of PAS has been to employ a prolonged or constant intravenous drip of dilute solutions of the sodium salt of para-aminosalicylic acid (sodium PAS) (6). Löffler and Moeschlin (7) have reported the use of intravenous sodium PAS in doses of 40 gm. per day (approximately 30 gm. of PAS) for periods up to six weeks in the therapy of tuberculous meningitis, utilizing a continuous intravenous drip with 5 mg. of heparin added to each liter of solution. The purpose of the present communication is to describe an experience with the subcutaneous administration of large volumes of dilute solutions of sodium PAS utilizing lyophilized hyaluronidase[3] to facilitate the absorption of the fluid (8, 9).

METHOD

In recent months a lyophilized powder of sodium PAS[4] has been employed. This material is available in sterile vials containing 6.25 gm. of sodium PAS equivalent to 5 gm. of free PAS. The powder was readily dissolved by the addition of 15 to 30 cc. of the solution to be used for hypodermoclysis and formed a clear straw-colored solution. This solution was then

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[2] This investigation was supported by a research grant from the National Institutes of Health, Public Health Service.

[3] Hyaluronidase supplied as "Wydase" by Wyeth Co., Philadelphia.

[4] Sodium PAS supplied as "PASNA Lyophilized" by Barnes Hind Laboratories, San Francisco.

added to the conventional large containers of 5 per cent dextrose in distilled water. Five per cent dextrose in distilled water was employed because each 18.75 gm. of sodium PAS (15 gm. PAS) contains 3.75 gm. of sodium, and the administration of large amounts of sodium would have been contraindicated in patients who have had pulmonary resection. Isotonic saline, distilled water, or 5 per cent dextrose in saline may be employed if desired. For intravenous administration, concentrations of 3 or 4 per cent of PAS are satisfactory. For subcutaneous administration the concentration of PAS should not exceed 2 per cent, and 1.5 per cent or 1.0 per cent concentrations produce minimal tissue irritation. (The concentration was calculated on the basis of 5 gm. of PAS per vial rather than 6.25 gm. of sodium PAS.) To facilitate absorption of fluid, a hyaluronidase preparation frequently was added at the start of the subcutaneous infusions. This hastened fluid uptake (as measured by comparing one leg without, and one leg with, hyaluronidase), and the procedure was continued subsequently when plasma PAS concentrations were determined. The hyaluronidase was injected into the tubing near the site of subcutaneous injection with a hypodermic needle in amounts of 60 to 150 turbidity-reducing units if one thigh was used and 150 turbidity-reducing units if the hyaluronidase was divided between the two thighs. In general, the lateral region of one thigh was used for each infusion with an intravenous drip set, but the hypodermoclysis may be given into both thighs or into the medial aspect of the thigh.

For the studies in which the concentration of PAS in the blood was determined, the method of administration was as follows: Sodium PAS (6.25 to 12.5 gm.) was given intravenously or subcutaneously in 1.5 or 2 per cent solution over a constant period (two hours) and oxalated blood specimens were obtained for determinations after one, two, three, four, and six hours. With one exception,[4] the patients in whom the blood PAS concentrations were determined had not received PAS within the preceding sixteen hours. For intravenous studies an antecubital vein was employed, and for subcutaneous studies the sodium PAS and 60 units of hyaluronidase were injected into one thigh. Blood specimens were refrigerated until determinations were made. All determinations were made within twenty-four hours by the method of Marshall (10), using reagents not more than one week old. Twelve studies were made on 10 patients hospitalized for tuberculosis. In addition, the PAS concentrations in the blood were determined one, two, and four hours after ingestion of 4 gm. of sodium PAS in 7 patients who were receiving 4 gm. of sodium PAS orally three times each day during their hospitalization. These determinations were made after the second of the three daily doses to permit comparison of the PAS concentrations obtained following administration of the drug by the oral route with those obtained by the parenteral routes.

RESULTS

Concentrations of PAS attained in the blood: The concentrations of PAS in the blood are shown in table 1. The concentrations attained following subcutaneous administration were of the same general magnitude as those obtained when an equal dose was given intravenously. The peak concentrations were somewhat lower, occurred later, and the concentrations at six hours were slightly higher than those obtained by using the intravenous route.

The plasma PAS concentrations of the patients who received 4 gm. oral doses are indicated in table 2. As may be seen, the peak values were found early and were slightly higher than those obtained with 6.25 gm. subcutaneously.

Continued subcutaneous administration: In addition to administration of subcutaneous sodium PAS for the pharmacologic studies, in 16 instances following thoracic surgery sodium PAS was administered by the subcutaneous route for

[4] In this patient the concentration of PAS in the blood at the start of the experiment was 1 mg. per 100 ml.

one to fourteen days, with satisfactory tolerance by the patients. In 3 cases of tuberculous meningitis, subcutaneous sodium PAS was given continuously for as long as thirty-four days in doses of 39.5 gm. of sodium PAS per day and, after a brief rest period, was continued for a total of forty-six days of therapy.

Untoward reactions: Local reactions were encountered but were mild. A sensation of burning at the site of injection occurred when the sodium PAS solution was given too rapidly or was above a 2 per cent concentration. The burning sensation was of brief duration when the rate of administration was decreased.

TABLE 1

Concentrations of PAS in the Blood After Parenteral Administration of Sodium PAS*

DOSE	TIME FOR ADMINISTRATION	ROUTE	HOURS AFTER START OF ADMINISTRATION LEVELS IN MG. PER 100 ML.				
			1	2	3	4	6
6.25 gm. (5 gm. PAS)	2 hours	Subcutaneous	4.1	7.8	6.9	5.1	2.5
		Intravenous	10.6	12.1	8.2	4.0	1.3
12.5 gm. (10 gm. PAS)	2 hours	Subcutaneous	10.8	21.5	23.1	14.3	10.7
		Intravenous	27.3	31.7	20.6	12.7	6.3

* Represents average of values obtained for each dose and route in 3 patients.

TABLE 2

Concentrations of PAS in the Blood After Oral Administration of Sodium PAS*

DOSE	ROUTE	HOURS AFTER ADMINISTRATION IN MG. PER 100 ML.			
		1	2	3	4
4 gm.	Oral	9.1	7.0	—	3.0

* Represents average of values obtained in 7 patients following the second of three daily doses of 4 gm. of sodium PAS.

Areas of swelling, 2 to 4 cm. in diameter, accompanied by redness and tenderness were seen occasionally at the site of the injection after single injections. These reactions subsided twenty-four to seventy-two hours after discontinuance of sodium PAS. When sodium PAS was repeatedly administered by the subcutaneous route, tenderness always appeared but was never sufficiently severe to require the use of analgesic drugs, and only with prolonged administration have rest periods been necessary.

One patient had previously had a generalized reaction to oral sodium PAS, consisting of apprehension, tightness of chest, uncontrollable weeping, and heaviness of arms and legs. Sodium PAS was given to this patient in gradually increasing doses by the intramuscular and oral routes until a tolerance to 4 gm. three times per day, orally, was obtained. The plasma concentration of PAS two

hours after ingestion of a 4 gm. dose was 5 mg. per 100 ml. When sodium PAS was then given to this patient by the subcutaneous route, she developed all of the symptoms she had initially experienced with oral sodium PAS except the heaviness of the limbs. The symptoms were most severe when the plasma concentration of PAS approached 30 mg. per 100 ml. The acute symptoms subsided in one to three hours but the patient was uncomfortable for the remainder of the day. It is believed that the high concentrations of PAS in the blood exceeded the concentrations to which she had been accustomed; hence, produced anew her symptoms of intolerance to sodium PAS.

DISCUSSION

On the basis of the above observations, it is believed that the subcutaneous administration of sodium PAS is a practicable method to supplement or replace oral or intravenous routes in instances where intensive therapy is indicated. In general, oral medication is satisfactory because of patient tolerance, economy, and ease of administration. The use of parenteral routes of administration is better suited during critical periods. The higher concentrations of PAS attained in the blood following parenteral administration should provide better diffusion gradients into the cerebrospinal fluid and, presumably, into tuberculous tissues and areas of surgical trauma.

Following subcutaneous administration with hyaluronidase, concentrations of PAS are attained in the blood which are comparable to those attained with intravenous administration and are two to three times those (11) attained following administration of the usually tolerated oral doses. The delayed peak and higher concentrations six hours after administration, noted following subcutaneous administration, suggest a depot effect. Following oral administration, higher concentrations of PAS appear in the blood earlier than they appear following subcutaneous administration, but the maximum concentration that can be achieved by the oral route is less because the dose which can be tolerated is limited. Following oral administration, cerebrospinal fluid PAS determinations reveal only traces or small amounts of the drug. After subcutaneous administration, PAS concentrations in the cerebrospinal fluid as high as 14.1 mg. per 100 ml. were observed. From these limited observations, it appears that the peak cerebrospinal fluid concentration lags behind the concentration of drug in the blood by apparently two hours. The drug persists longer in the cerebrospinal fluid, however, and in the present study appreciable concentrations of PAS were detected in the cerebrospinal fluid for ten to twelve hours after subcutaneous administration of 18.75 gm. of sodium PAS.

The parenteral routes have the advantage of being available regardless of gastrointestinal tolerance or state of cooperation of patients, but the primary objective in their use is to produce high concentrations of PAS in the blood or in the cerebrospinal fluid. The intravenous route is not particularly bothersome to the patient if adequate support for the extremity is provided, sufficient patent veins are present, and excessive motion of the limb is unnecessary. Intravenous therapy requires more medical attention, demands more time for proper control,

produces more interference with postoperative care, is difficult to use over extended periods of time, and often is virtually impossible in infants.

Conversely, subcutaneous administration of PAS is generally well tolerated locally, is easy to administer, requires little attention, produces minimal impediments to postoperative nursing care, and probably can be used almost universally in infants. The subcutaneous method can be alternated with intravenous administration if prolonged parenteral treatment is desired, thus providing rest periods for both veins and subcutaneous tissues.

Tentatively, it is recommended that for most situations requiring parenteral PAS 12.5 to 18.75 gm. of sodium PAS (10 to 15 gm. of PAS) in a 1.5 per cent solution be given subcutaneously over a three-hour period, twice daily, eight hours apart. We have used 6.25 to 39.5 gm. of sodium PAS (5 to 30 gm. of PAS) per day. Some flexibility in dosage is necessary to provide the desired intensity of therapy.

SUMMARY

The sodium salt of para-aminosalicylic acid (PAS) may be given subcutaneously in solution of 1 to 2 per cent concentration in dosage of 6.25 to 39.5 gm. per day (with hyaluronidase). The concentrations of PAS attained in the blood are comparable to those noted after intravenous administration of sodium PAS.

Subcutaneous administration of sodium PAS is a safe, effective, well-tolerated method of PAS administration and has the advantages of ease of administration and applicability to infants, as well as permitting rest periods for veins when parenteral PAS therapy is desired over extended periods.

Cerebrospinal fluid PAS concentrations of 14 mg. per 100 ml. have been observed following the subcutaneous administration of 12.5 to 18.75 gm. of sodium PAS.

Local reactions can be minimized by slow administration of PAS solutions which do not exceed a 2 per cent concentration.

Parenteral sodium PAS should be given with caution to patients who have experienced systemic reactions to PAS administered by the oral route and to patients with impaired renal function.

SUMARIO

La Administración Subcutánea de la Sal Sódica del Acido Para-Aminosalicílico en el Tratamiento de la Tuberculosis

La sal sódica del ácido para-aminosalicílico puede ser administrada subcutáneamente en solución de una concentración de 1 a 2 por ciento a dosis diarias de 6.25 a 39.5 gm. (con hialuronidasa). Los tenores sanguíneos obtenidos son comparables a los conseguidos con la administración endovenosa del PAS sódico.

La administración subcutánea del PAS sódico constituye un método inocuo, eficaz y bien tolerado para suministrar PAS, poseyendo las ventajas de fácil administración, aplicabilidad a las criaturas y de ofrercer períodos de descanso

a las venas cuando se desea el uso parentérico del PAS durante períodos prolongados de tiempo.

A continuación de la inyección subcutánea de 12.5 a 18.75 gm. de PAS sódico, obtuviéronse concentraciones de 14 mg. de PAS por 100 ml. en el líquido cefalorraquídeo.

Pueden reducirse al mínimo las reacciones locales con la administración lenta de soluciones que no excedan una concentración de 2 por ciento.

Hay que administrar con cautela el PAS sódico por vía parentérica a los enfermos que han manifestado reacciones generales al PAS por vía oral y a los que padecen de disfunción renal.

RESUME

Administration sous-cutanée du sel de sodium de l'acide para-amino salicylique dans le traitement de la tuberculose

Le sel de sodium de l'acide para-aminosalicylique peut être administré par la voie sous-cutanée sous la forme d'une solution à 1% ou à 2%, à la dose de 6,25 à 39,5 g. par jour (avec adjonction d'hyaluronidase). Les concentrations sanguines obtenues sont comparables à celles réalisées par l'administration intraveineuse de PAS sodique.

L'administration sous-cutanée du PAS sodique est un mode d'administration sûr, efficace et bien toléré; elle offre l'avantage d'être applicable au nourrisson et elle permet la mise au repos des veines quand on désire poursuivre pendant longtemps l'administration parentérale du PAS.

Dans le liquide céphalo-rachidien, une concentration du PAS de I4 mg. pour I00mml. a été obtenue à la suite de l'administration sous-cutanée de 12,5 à I8,75 g. de PAS sodique.

Les réactions locales peuvent être minimisées par l'administration de solutions dont la concentration ne dépasse pas 2%.

L'administration parentérale de PAS sodique doit être prudente chez les sujets ayant présenté des réactions générales lors de l'administration orale du PAS ainsi que chez ceux dont la fonction du rein est altérée.

REFERENCES

(1) The Therapeutic Trials Committee of the Swedish National Association against Tuberculosis: Para-aminosalicylic acid treatment in pulmonary tuberculosis: Comparison between 94 treated and 82 untreated cases, Am. Rev. Tubere., 1950, *61*, 597.

(2) Lehman, J.: The treatment of tuberculosis in Sweden with para-aminosalicylic acid (PAS): A review, Dis. of Chest, 1949, *16*, 684.

(3) Warner, O. M.: Effect of varying PAS dosage in combined SM-PAS therapy for pulmonary tuberculosis, Minutes of Eighth Streptomycin Conference of the Veterans Administration, Nov. 10, 1949, p. 112.

(4) Tempel, C. W., Hughes, F. J., Jr., Mardis, R. E., Towbin, M. N., and Dye, W. E.: Combined intermittent regimens employing streptomycin and para-aminosalicylic acid in the treatment of pulmonary tuberculosis, Minutes of Ninth Streptomycin Conference of the Veterans Administration, April 18, 1950, p. 36.

(5) Davin, J. R., and Rogers, A. E. T.: Febrile reactions to para-aminosalicylic acid, Am. Rev. Tubere., 1950, *61*, 643.

(6) Paraf, J., Desbordes, J., Paraf, M., and Zivy, P.: El tratamiento de la tuberculosis por el acido paraaminosalicilico, Hoja tisiol., 1950, *10*, 135.
(7) Löffler, W., and Moeschlin, S.: Resultate der kombinierten Behandlung der Meningitis Tuberculosa mit Streptomycin und Paraaminosalicylsäure (PAS) im Vergleich zur Behandlung mit Streptomycin allein, Schweiz. med. Wchnschr., 1950, *80*, 365.
(8) Schwartzman, J.: Hyaluronidase in pediatrics, J. Pediat., 1949, *34*, 559.
(9) Jaworski, A. A., and Farley, J. E., Jr.: Hyaluronidase in administration of fluids, Am. J. Dis. Child., 1950, *79*, 59.
(10) Marshall, E. K., Jr.: Determination of para-aminosalicylic acid in blood, Proc. Soc. Exper. Biol. & Med., 1948, *68*, 471.
(11) Duncan, D. K., Carr, D. T., Pfuetze, K. H., and Power, M. H.: Serum concentrations of para-aminosalicylic acid (PAS) produced by various forms of PAS, Dis. of Chest, 1951, *19*, 138.

Case Reports

THE DEVELOPMENT OF TUBERCULOUS MENINGITIS FOLLOWING CORTISONE THERAPY[1]

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INTRODUCTION

The increasingly widespread use of cortisone[2] makes a critical appraisal of its possible effects a matter of considerable importance at the present time. In a previous report (1), attention was called to the development of urinary tract infections in two patients receiving cortisone. The circumstances under which these complications occurred appeared more than coincidental, and it was postulated that at least in some patients there might exist a disturbance of immune body mechanisms as a result of cortisone therapy.

The role of cortisone or adrenocorticotropic hormone (ACTH) in regard to bacterial infections has not yet been clearly defined. It is of interest that the use of ACTH in patients with pneumococcal pneumonia was shown by Kass, Ingbar, and Finland (2) to induce profound changes in clinical symptoms without demonstrably affecting the etiologic agent and antibody production. Other investigators (3) have reported similar findings in a variety of bacterial infections. Cortisone and/or ACTH thus appear to be effective in aborting the inflammatory reactions associated with certain acute bacterial infections. In the writers' experience, however, some patients seem unable to respond adequately to bacterial infections arising during or shortly following prolonged cortisone therapy.

Several recent reports have recorded the effect of cortisone or ACTH on tuberculous lesions. Hart and Rees (4) demonstrated in the mouse enhanced susceptibility to tuberculosis following cortisone administration. They pointed out that, although human tuberculosis differs considerably from murine, tuberculosis in man may possibly be similarly aggravated. Subsequently, Spain and Molomut (5) demonstrated that in guinea pigs experimentally infected with tuberculosis and treated, respectively, with saline solution as a control, cortisone, streptomycin, and cortisone combined with streptomycin, the lesions in those animals receiving cortisone were more extensive, more widely distributed, and less well organized histologically than the lesions in the animals which received no hormone. Although Hench and co-workers (6) utilized cortisone in the treatment of tuberculous arthritis in humans, they note that its use in cases of tuberculosis should be investigated with caution. Freeman, Pershing, Wang, and Smith (7) administered ACTH to two patients with pulmonary tuberculosis, with definite spread of the lesion in one and questionable extension in the other. In a more recent investigation with

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[2] Cortisone was made available through a special grant from the Division of Hospitals, Public Health Service, Federal Security Agency.

ACTH (8), no definite evidence of a deleterious effect upon tuberculous lesions in humans was noted.

At the present time, therefore, there is evidence which suggests that, at least in some instances, cortisone may be deleterious to the body's ability to handle tuberculous infection. Clinical and pathologic studies, however, following prolonged cortisone therapy in patients with unsuspected tuberculosis have thus far not been reported. The purpose of the present communication is to report a case of tuberculous meningitis which developed shortly after the cessation of cortisone administration.

CASE REPORT

S. S., a 44-year-old white female, was admitted to the U. S. Marine Hospital, Staten Island, New York, on April 17, 1950. The diagnosis of relapsing febrile nodular nonsuppurative panniculitis (Weber-Christian disease) had been established during the first of five previous admissions here, in February 1948. A typical history and clinical course of this condition had been noted, as well as histopathologic confirmation of the diagnosis on biopsy of one of the nodules of the left lower extremity. Examination of biopsy material showed mild diffuse chronic inflammatory and necrobiotic changes in the fibroblastic connective tissue and adjacent fat. Mild infiltration of neutrophils was noted. There were small foci of necrosis without caseation and an occasional giant cell. Marked constriction of small blood vessels suggested a panniculitis which was probably due to vascular obliteration resulting in a foreign-body-type granuloma. Cultures taken from the nodule showed no growth on aerobic and anaerobic media. When autopsy findings revealed the presence of tuberculous lesions, the slides of the biopsy material were reviewed. No acid-fast organisms could be demonstrated on smear and there was some difference of opinion among the pathologists as to whether the histologic findings were those of Weber-Christian disease or of tuberculosis. Erythema nodosum or erythema induratum was considered in retrospect by one pathologist who regarded small areas of necrosis and neutrophilic infiltration as contraindications to the use of the term "nonsuppurative nodular panniculitis." In November 1948 a biopsy specimen of another similarly located typical nodule was examined by Dr. G. F. Machacek of the Dermatology Department of Columbia University, College of Physicians and Surgeons, who reported a diagnosis of nonsuppurative panniculitis.

Since the beginning of her illness, the patient continued to have an intermittently febrile course, with bouts of migratory joint pains and recurrent painful subcutaneous nodules of the lower extremities, with only partial relief by the constant use of antipyretics and analgesics. The erythrocyte sedimentation rate remained elevated to 29 mm. per hour (Cutler method). Multiple blood cultures were negative for aerobic and anaerobic organisms. Roentgenograms of the chest repeatedly showed no evidence of tuberculosis. The patient produced no sputum for examination and the cutaneous reaction to tuberculin was not tested. In view of her persistent and prolonged disability despite the use of a wide variety of therapeutic agents, a trial of cortisone therapy was initiated.

Cortisone administration: Beginning May 5, 1950 cortisone acetate (100 mg.) was given intramuscularly daily for five days; for the next six days the dosage was increased to 200 mg. daily, and thereafter an average of 100 mg. daily was given until June 10, 1950. A total of 3.635 gm. of cortisone acetate was given intramuscularly over a thirty-five-day period. Except for the development of a "moon facies" on the tenth day of treatment and moderate acne on the twentieth day, no other untoward effects of cortisone therapy were observed. Although marked subjective improvement in sense of well being and appetite was noted, the patient's febrile course was unaffected and transient joint pains recurred intermittently. No further outcroppings of subcutaneous nodules occurred during this hospitalization. The patient was discharged June 23, 1950 to continue convalescence at home.

Appearance of meningitis: The patient returned for her seventh and final admission July 3, 1950, complaining of severe frontal headache, weakness, and fever (progressive in nature)

for the past week. The day prior to admission, her temperature was 104°F. and she became drowsy and intermittently disoriented.

Physical examination revealed a rectal temperature of 99.8°F. and scattered small ecchymotic areas over the surface of the left leg; there were no other abnormal findings. The following day nuchal rigidity and hyperactive deep tendon reflexes appeared, as well as ankle clonus and positive left Hoffman and Babinski signs. The patient's rectal temperature was 104°F. A total leukocyte count showed 14,500 cells per cu. mm. with 83 per cent neutrophils. Cerebrospinal fluid examination revealed an initial pressure of 477 mm. of water, with normal manometrics, and 980 cells per cu. mm., predominantly neutrophils; protein, 100 mg. per 100 cc.; chlorides, 114 milliequivalents per liter; the quantity of fluid was insufficient for sugar determination. A pedicle did not form. A culture of the initial cerebrospinal fluid showed gram-negative cocco-bacilli, resembling *Hemophilus influenzae*. Subcultures were unsuccessful, however, and all subsequent cerebrospinal fluid specimens showed no growth on culture for aerobes and anaerobes.

Repeated urinalyses were normal. The hemogram consistently showed a moderate normochromic anemia and slight leukocytosis with neutrophilia, similar to that noted on admission. An electrocardiogram was normal. Determinations of the serum electrolytes, nonprotein nitrogen, and fasting blood glucose revealed normal values. An upright posterior-anterior roentgenogram of the chest revealed no pulmonary pathology, nor was there *any change* when compared with previous films. Roentgenograms of the skull, cervical spine, and abdomen were normal except for some generalized demineralization of the pelvic bones and spine.

The patient received 200,000 to 300,000 units of penicillin intramuscularly daily from July 4, 1950, until July 29, 1950. On three occasions during the first week, 10,000 units of penicillin were given intrathecally. Chloramphenicol (Chloromycetin), 1 gm. every six hours, was also given over the same twenty-five-day period. Sulfadiazine (5 gm. in 2,000 cc. of 10 per cent glucose) was given the day following admission and was changed to 1 gm. orally every four hours for the next four days. From July 8 until July 19, 1950 streptomycin in dosage of 0.5 gm. every six hours was given. Although the cerebrospinal fluid protein remained elevated at 200 mg. per 100 cc., the cell count returned to normal by July 11. Complement fixation tests for encephalitis and choriomeningitis were negative.

The patient continued to have intermittent rises of fever to 104°F., as well as intermittent periods of lucidity and disorientation. On August 7 neurologic examination disclosed a left Horner's syndrome, marked rigidity of the neck, spastic flexion of both elbows, and absent triceps jerks bilaterally. The rest of the deep tendon reflexes were hyperactive, with bilateral ankle and patellar clonus and an extensor plantar response on the left. It was the neurologist's impression that a cervical cord syndrome was present, following subsidence of an acute meningeal infection.

Except for minor alterations in the patient's sensorium and some increased nuchal rigidity, her course was unchanged during the next ten days. On August 17 the patient became comatose and remained so until she quietly died August 21, 1950.

Post-Mortem Examination

Macroscopic examination: At autopsy the body was pale; hypertrichosis was present over the chin. Lymph nodes in the abdominal and thoracic cavities were found to be discrete, rubbery, and enlarged to 3 cm. in diameter. On cut surface the lymph nodes were mottled yellowish-tan, and no material could be scraped away. In the periphery of the right upper lobe of the lung an area of consolidation was found that measured approximately 4 cm. in diameter, was poorly demarcated from surrounding tissue, and on section revealed a yellow, dry, caseous center which when scraped away left a rough granular tissue behind. Other small nodules found in this lobe were irregular in outline, gray-red, dry, and firm. No caseation was evident in these nodules. The remaining lobes were subcrepitant but free

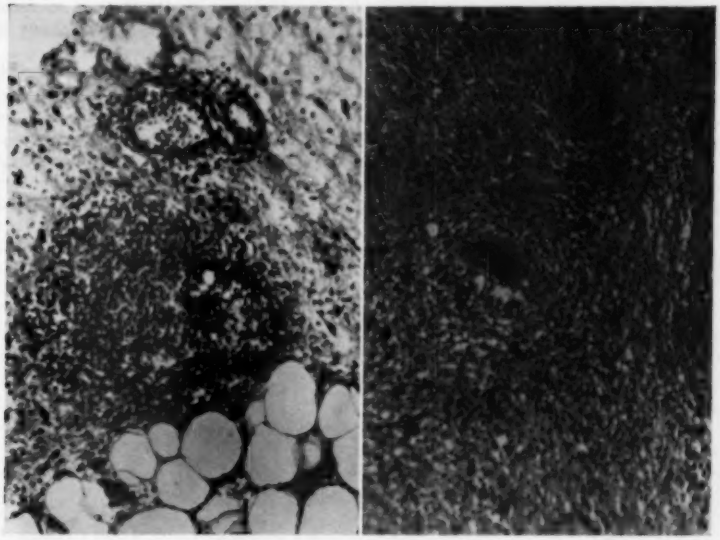

FIG. 1. (Left) Biopsy subcutaneous panniculitis nodule; necrosis and suppuration.
FIG. 2. (Right) Tuberculous lung. Healing with scarring and giant-cell formation.

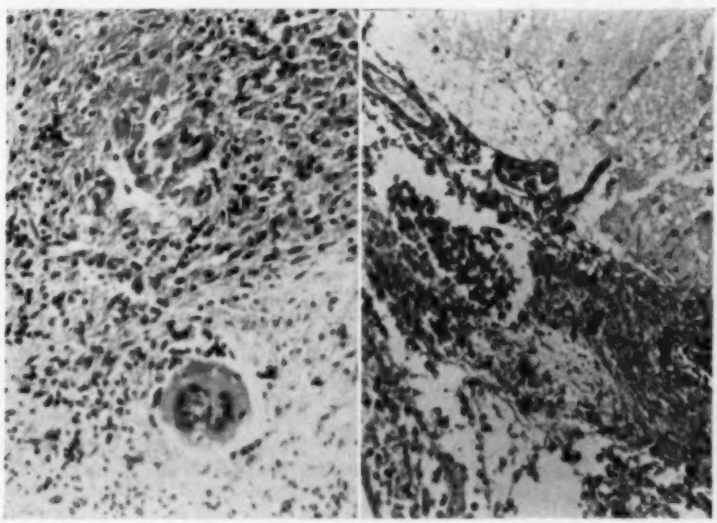

FIG. 3. (Left) Tuberculous ileum. Extensive productive tuberculosis.
FIG. 4. (Right) Tuberculous meningitis. Lymphocytes and monocytes enmeshed in fibrinous exudate.

of nodules. The terminal ileum and a few centimeters of cecum showed blotchy, reddened, flat mucosal patches which were not ulcerated, caseous, or thickened. Lymphoid tissue did not appear hypertrophied.

The brain showed a gelatinous translucency over the hemispheres and a moderate flattening of the convolutions. The pia and arachnoid at the base of the brain showed thick, milky, gray-white exudate about the structures in this area. The exudate was continuous over the cervical cord but less thick. The brain was moderately edematous on surface made by sectioning. The fluid in the ventricles was clear. No tubercles were seen. The adrenal glands were normal in size, shape, and consistency, with well-defined architectural pattern. The pituitary was grossly normal. No gross lesions were visible in any other organ. No subcutaneous nodules were present.

Bacteriologic studies: Tubercle bacilli were demonstrated in the meningeal exudate and periaortic abdominal lymph nodes by direct smear, culture, and inoculation of a guinea pig.

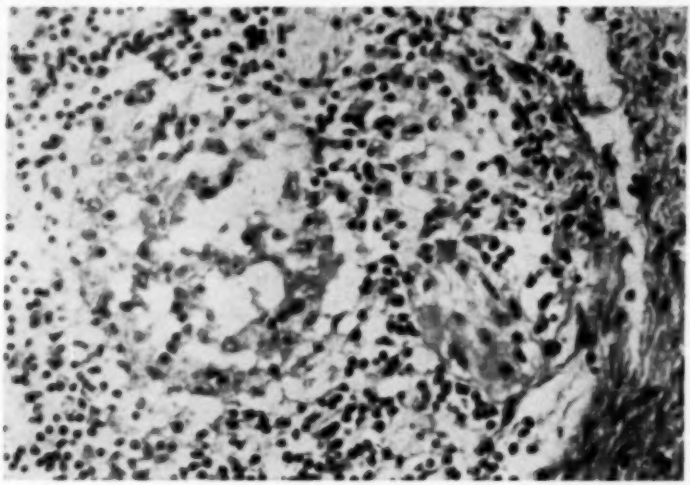

FIG. 5. Epithelioid and giant-cell production in tuberculous meningeal exudate.

The site of injection in the guinea pig's groin was unusually rubbery and noncaseous grossly, and to this extent the animal and human lesions were similar. Smears later from preserved lung specimens showed acid-fast organisms; smears of ileum and cecum did not.

Microscopic examination: On microscopic examination, many caseous tubercles were seen in the necrotic nodule in the right upper lobe of the lung, in many of the pneumonic-like areas in the same lobe, in the patchy mucosal areas in the ileum and cecum, and in the lymph nodes generally; the meningeal exudate showed masses of lymphocytes and monocytes with some tubercle formation. The adrenal gland showed a small area of lymphocytic infiltration without necrosis in the zona reticularis.

The histologic morphology of the lesions varied throughout each of the involved organs. Some pulmonary lesions showed "soft" focal necrosis, fibrinous exudate with many lymphocytes and monocytes, while other pulmonary lesions showed "hard" scarred tubercles with a fibrotic rim of granulation tissue and thick-walled small blood vessels, a few chronic inflammatory cells, and large Langhans' type of giant cells. The surrounding parenchyma showed emphysema of some alveoli, fibrosis in the alveolar walls, and cuboidal metaplasia

of the lining cells. The tubercles in the lymph nodes showed various stages of healing and regression in the same and different nodes. The intestinal tubercles also showed fibrosis, epithelioid cells, and giant cells, all of which seemed to indicate an old granulomatous process with lesions in progressive stages of fibrosis or proliferation.

The meningeal exudate showed no old fibrotic lesions, but consisted of many lymphocytes and monocytes enmeshed in abundant fibrin. Focal necrosis occurred in some areas, and a fair number of epithelioid cells and giant cells were seen. There was little fibroblastic activity or vascularity. Throughout all lesions and even in vascular lumina there was a conspicuous absence of neutrophils.

DISCUSSION

A case with the diagnosis of Weber-Christian disease has been presented in which, prior to and during the administration of cortisone, there was no roentgenographic evidence to suggest the presence of a tuberculous infection. Despite the absence of therapeutic response of the Weber-Christian disease to cortisone, no serious untoward effects were observed until twenty-four days after cessation of therapy, when signs of meningitis appeared. The etiology of the meningitis was not definitely established during life. Postmortem examination, however, disclosed tuberculosis involving the meninges, lung, ileum, cecum, and lymph nodes.

It cannot be denied that the administration of cortisone and the subsequent appearance of tuberculous meningitis in this patient may have been entirely fortuitous, but the course of events suggested a more than coincidental relationship.

While the influence of cortisone on immune body mechanisms and resistance to infections has not been defined with certainty, recent reports (9) have stressed the probable role of this steroid in these relationships. This would appear to be of importance not only in tuberculous infection but in other bacterial infections as well, as noted in our previous experience and those of others (1, 2, 3).

While interference with the operation of immune mechanisms conceivably could explain the development of tuberculosis in the case presented, still another possibility remains to be considered. It has been shown experimentally (10) that the adrenocorticotropic activity of the anterior pituitary is influenced by the tissue level of the oxysteroids. When tissue demands have been satisfied through adequate therapy, the response to stress is optimal. After tissue saturation of oxysteroids has been achieved, adrenocorticotropic output of the anterior pituitary is reduced. The writers have observed, as have other investigators (11), that after prolonged cortisone administration in some patients there is evidence of adrenal and anterior pituitary depression, depression of urinary 17-ketosteroids, and diminished response to the pituitary ACTH test. Depending on the individual tissue requirements and response, a state is reached in which the adrenal cortex and anterior pituitary become insensitive to further stimulation and the organism is unable to adjust to further demands made upon it. It may be hypothesized that, at this stage of therapy, tissue needs, created by various infectious agents, for increased adrenal corticoids and other hormones elaborated by the anterior pituitary cannot be satisfied.

It is believed that such a mechanism may have existed in the case reported and might have influenced the course of the tuberculous infection.

Regardless of the mechanism involved, it appears that the development of tuberculosis and other bacterial infections during or following the long-term administration of cortisone is a potential hazard deserving consideration. Continued careful clinical observation is necessary to decide ultimately the relative importance of this factor in cortisone therapy. Pathologic review of cases should consider that, because of the effect of cortisone on mesenchymal tissues, careful attention must be given to the quantity and quality of

inflammatory cell response, fibroblastic activity, decreased vascularity, and delayed granulation tissue response if we are to ascertain the possible effect of this adrenocorticosteroid. In the present case, however, there was no evidence that the nature of the individual tuberculous lesions had been altered as a result of the cortisone administration.

SUMMARY

A case of Weber-Christian disease with unsuspected tuberculosis is presented in which cortisone was administered and was followed by tuberculous meningitis and death.

SUMARIO

La Cortisona en la Tuberculosis: Historia Clínica

Preséntase un caso de mal de Weber-Christian con tuberculosis insospechada en el cual la administración de cortisona fué seguida de meningitis tuberculosa y la muerte.

RESUME

La Cortisone Dans la Tuberculose: Présentation d'un cas

L'auteur présente un cas de maladie de Weber-Christian avec tuberculose insoupçonnée dans lequel l'administration de cortisone a été suivie de méningite tuberculeuse et de mort.

REFERENCES

(1) Doerner, A. A., Naegele, C. F., Regan, F. D., Shanaphy, J., and Edwards, W.: The effect of cortisone administration on sensitized RH-negative pregnant women and an erythroblastotic infant: A report of three cases, J.A.M.A. (to be published).

(2) Kass, E. H., Ingbar, S. H., and Finland, M.: Effects of adrenocorticotrophic hormone in pneumonia: Clinical, bacteriological and serological studies, Ann. Int. Med., 1950, *33*, 1081.

(3) (a) Reeder, W. H., and Mackey, G. S.: Nebulized cortisone in bacterial pneumonia, Dis. of Chest, 1950, *18*, 528.

(b) Finland, M., Kass, E. H., and Ingbar, S. H.: Effects of ACTH in primary atypical (viral) pneumonia and in pneumococcal pneumonia, Proceedings of First Clinical ACTH Conference, The Blakiston Co., Philadelphia, 1950, p. 529.

(4) Hart, P. D'A., and Rees, R. J. W.: The enhancing effect of cortisone on tuberculosis in the mouse, Lancet, 1950, *2*, 391.

(5) Spain, D. M., and Molomut, N.: Effects of cortisone on the development of tuberculous lesions in guinea pigs and on their modification by streptomycin therapy, Am. Rev. Tuberc., 1950, *62*, 337.

(6) Hench, P. S., Kendall, E. C., Slocumb, C. H., and Polley, H. F.: Effects of cortisone acetate and pituitary ACTH on rheumatoid arthritis, rheumatic fever and certain other conditions: A study in clinical physiology, Arch. Int. Med., 1950, *85*, 545.

(7) Freeman, S., Pershing, J., Wang, C.C., and Smith, L. C.: The effect of ACTH on patients with pulmonary tuberculosis, Proceedings of First Clinical ACTH Conference, The Blakiston Co., Philadelphia, 1950, p. 509.

(8) (a) Tompsett, R., LeMaistre, C., Muschenheim, C., and McDermott, W.: Effects of ACTH on tuberculosis in humans, J. Clin. Investigation, 1950, *29*, 849.

(b) LeMaistre, C., Tompsett, R., Muschenheim, C., Moore, J. A., and McDermott, W.: Effects of adrenocorticotropic hormone and cortisone in patients with tuberculosis, J. Clin. Investigation, 1950, *30*, 445.

(9) WHITE, A.: Role of the adrenal cortex in immunity, J. Allergy, 1950, *21:* 273. Relation of adrenals to immunity, Bull., N. Y. Acad. Med., 1948, *24:* 26.
(10) SAYERS, G.: Pituitary Regulation of Adrenal Cortical Activity, Ch. IV, Soskin's *Progress in Clinical Endocrinology*, Grune and Stratton, Inc., New York, 1950.
(11) SPRAGUE, R. G., POWER, M. H., AND MASON, H. L.: Physiological effects of cortisone and pituitary adrenocorticotrophic hormone (ACTH) in man, J.A.M.A., 1950, *144*, 1341.

LIPOID PNEUMONIA[1,2]

A Report of Two Cases

HERMAN WEISSMAN

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Lipoid pneumonia, as described in the medical literature of recent years, has been found to be due to the aspiration of oily material. Most of the cases were attributable to the medicinal use of mineral oil and of cod-liver oil, milk, and egg yolk while several cases of lipoid pneumonia in children were due to accidental aspiration of kerosene and gasoline. In both World Wars I and II, oil pneumonitis was reported as a result of aspiration of waters contaminated with Diesel oil by survivors of torpedoed ships.

It is the purpose of the present communication to report one case dissimilar to any the writer has seen described, namely, a lipoid pneumonia caused by the industrial use of petrolatum products. A more characteristic case of lipoid pneumonia, previously diagnosed as pulmonary tuberculosis, is also reported in detail.

Case 1. (D. A. R.) The patient, a 49-year-old white male, was admitted to this hospital on October 17, 1949. He had been discharged from the armed services in 1918 with a diagnosis of chronic bronchitis. From 1918 until 1928 he had worked as a metal polisher and for the next five years was employed in a melting department of an aluminum plant. In 1933 he started work as a maintenance man in a large machine shop. A roentgenogram obtained in 1938 revealed abnormal pulmonary findings. Not until 1943, however, did he begin to complain of dyspnea of effort and increase in cough. In 1947 his cough became severe and expectoration was productive of two ounces of purulent material. He was then admitted to a tuberculosis hospital where sputum examinations were negative for tubercle bacilli, and the cutaneous reaction to tuberculin was negative. He was advised that he had no pulmonary tuberculosis. The patient returned to work and, in October 1948, was admitted to another tuberculosis hospital because of persistence of symptoms. A bronchogram failed to reveal evidence of bronchiectasis. One of 4 sputum concentrates was positive for tubercle bacilli, but sputum cultures and a guinea pig inoculation were "negative." He was discharged with the diagnosis of pulmonary fibrosis of unknown etiology.

On admission to this hospital the patient complained of severe cough and expectoration of copious amounts of mucoid and purulent material, of marked dyspnea of effort, and of chest pains; at this time he appeared very undernourished. Physical examination was essentially unrevealing except for chest findings which consisted of numerous post-tussic rales in both bases and moderate wheezing throughout both lung fields. Marked clubbing of the fingers and toes was also noted.

Intensive laboratory studies, consisting of four sputum concentrates examined on smears, two sputum cultures, and one guinea pig inoculation, were negative for tubercle bacilli and fungi. Skin reaction to tuberculosis was positive in second-strength dilution. The hemogram and urine were normal, and a serologic test for syphilis was negative. Roentgenographic examination of the chest (figure 1) revealed fibrotic and patchy areas throughout both lungs, with possible emphysematous blebs in the right upper lobe. Patchy densities were noted in both hilar areas which were markedly enlarged. The right costophrenic sinus was obliterated.

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[1] From the Medical Service, Veterans Administration Hospital, Castle Point, New York.

[2] Reviewed in the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the author are the result of his own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

Further interrogation of the patient revealed that his industrial duties had consisted of blasting, with a hose under 150 pounds of pressure, machine parts which had been immersed in oil. He had worn no mask as protection against the nebulized oil and, as the patient stated, the area in which he had worked was laden with particulated oils. He had never used any mineral oil for medicinal purposes and he had no difficulty in deglutition. A specimen of 75 cc. of sputum was centrifuged and the upper layers gave the appearance of a greasy

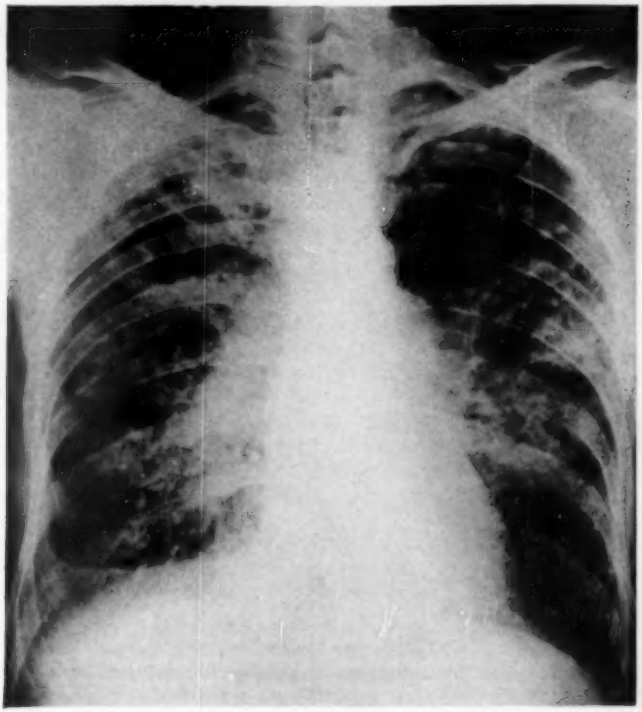

FIG. 1. Case 1 (D. A. R.). Chest roentgenogram revealing fibrotic and patchy infiltration throughout both lungs. Patchy densities are noted in both hilar areas which are markedly enlarged. The right costophrenic sinus is obliterated.

film. A smear of this film treated with Sudan IV was positive for fat. Still another centrifuged specimen with the appearance of a greasy film was treated with an acid-digestion mixture and no iodine vapors were noted. Repeated roentgenograms showed no essential change during the patient's stay at the hospital. The patient was discharged on November 8, 1949, with instructions to change his place of work and to work fewer than the eleven to twelve hours per day which he had been doing.

Comment: This is a case of pulmonary fibrosis of long standing, aggravated by inhalation of nebulized industrial oil over a period of several years, resulting in lipoid pneumonitis. Iodized oil injected for study apparently has disappeared entirely from the lungs as proved by the absence of iodine vapors.

Case 2. (A. G.) The patient, a white male of 54 years of age, was admitted to this hospital on November 5, 1946. He had been disabled with arthritis for eighteen years. In July 1946, he developed a moderate fever, and a chest roentgenogram revealed bilateral involvement which was interpreted at that time as "virus pneumonia." He obtained some relief from antibiotics. In August 1946, the patient again developed a moderate fever and his sputum at this time was found to be positive for *M. tuberculosis*.

The patient was moderately obese, had difficulty in walking, and was coughing severely, on occasion expectorating two cups of thin odorless sputum. Exhaustive sputum studies failed to confirm the previous finding of tubercle bacilli. Roentgenograms of the chest (figure 2) revealed irregularly circumscribed, patchy densities in the lower halves of both

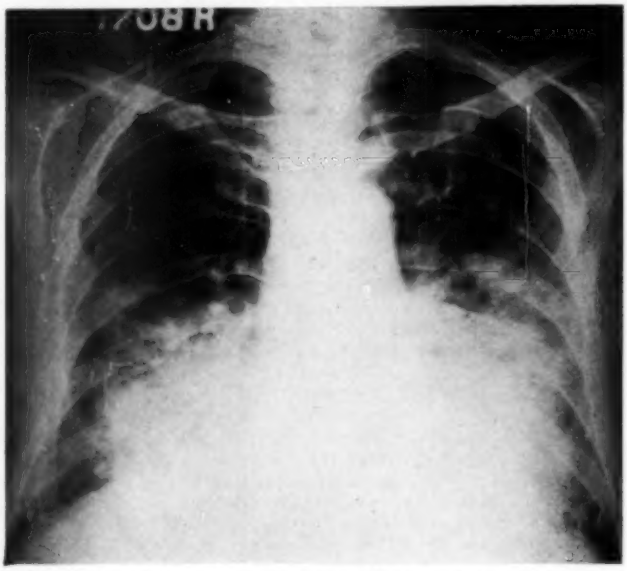

FIG. 2. Case 2 (A. G.) Roentgenogram of chest which reveals lower halves of both lung fields extending from the hilar areas to within 3 cm. of the outer lung margin.

lung fields extending from the hilar areas to within 3 cm. of the outer lung margin. A bronchoscopic examination revealed the tracheobronchial tree to be normal, and the aspirated material was negative for *M. tuberculosis*.

On further investigation it was found that the patient had difficulty in swallowing oily foods and that he had had severe bouts of coughing after eating fatty food, with resulting regurgitation. He had been using mineral oil for constipation for a number of years and stated that he very often aspirated milk. He thought that the food did not go down properly but was unable to localize the area of difficulty. With this information a search for oil globules in the sputum was made and their presence was demonstrated. Examination of material obtained by aspiration biopsy on the left lung on January 3, 1947 revealed evidence of lipoid pneumonia. During hospitalization the patient again had a febrile episode which was only slightly relieved by antimicrobial therapy but was greatly relieved by postural drainage. He improved somewhat and was transferred to a general hospital for further care of his arthritis and deglutitional defect.

Comment: This is the case of a debilitated and partly bedridden patient who, because

of difficulty in deglutition, had been repeatedly aspirating fatty material, with the result that he eventually developed lipoid pneumonia complicated by repeated superimposed infections.

COMMENT

Lipoid pneumonia is caused by aspiration into the lungs of various fatty materials of vegetable, animal, and mainly mineral origin. Mineral oil has been used for medicinal purposes since 1869. It was only in 1925, however, that Laughlen (1) reported the first four cases of lipoid pneumonia due to mineral oil. Numerous similar reports followed that publication but most of the cases reported to date have been based on post-mortem findings. It is important to point out that in several cases pneumonectomy has been performed for suspected neoplasms which have proved to be lipoid pneumonia.

The disease occurs in all ages; in infants because of forced feedings, and in adults because of internal or intranasal use of oils either by healthy persons or bedridden and debilitated patients. It is the continued use of the oil, even in small doses, which will result ultimately in an accumulation in the alveolar spaces of the lungs. It is not generally realized, however, that lipoid pneumonia may likewise occur as a result of aspiration of industrial mineral oils, which are less refined fractions in use for lubrication and cooling of machine parts. In this situation, the main cause is to be found in an oil spray produced by high air pressure in the cleaning of parts previously immersed in oil.

Presence of various oily substances in the alveoli produce various reactions. In 1928 Pinkerton (2) injected various oils into the lungs of experimental animals and found that the simple neutral vegetable oils produced no tissue reaction in the absence of infection. On the other hand, he found that animal fats caused fibrosis in the lumina of the alveoli and lymph nodes due to their hydrolytically produced fatty acids. The mineral oils were not hydrolyzed but were rapidly emulsified, producing a slow but inevitable fibrosis.

The reaction produced in the pulmonary alveoli are described as follows:

(1) Diffuse acute: interstitial proliferative inflammation with or without exudative pneumonia
(2) Diffuse chronic: representing the intermediate stage and consisting essentially of a proliferative fibrosis
(3) Chronic localized: representing tumor-like paraffinoma.

No studies of inhalation of nebulized oils were made until 1950, when Shashkas and associates (6) studied the fate of inhaled aerosols. Experimental mice were exposed to the mist of particulated liquid oils, and the initial concentration of retained oil droplets was similar for all the oil mists used. It was found that, after exposure to edible oil droplets (corn, peanuts, cod-liver oil), the concentration of retained oil decreased progressively during the succeeding ninety-six hours, while after exposure to liquid petrolatum and motor oil the concentration remained essentially unchanged. After heavy exposure for periods of two to four weeks, liquid petrolatum and motor oil droplet retention resulted in localized foreign body reaction of moderate severity with some patches of lipoid pneumonia.

For correct diagnosis, a detailed clinical and occupational history is of primary importance, with particular emphasis on usage of various oils and difficulty of deglutition. The symptoms of lipoid pneumonia depend mainly on the amount of aspirated oil and the patient's general condition. Increasing dyspnea and a productive cough, however, are the most important symptoms.

Abnormal findings on physical examination are not prominent. The principal roentgenographic findings, depending upon the duration and extent of the disease, are enlarged perihilar shadowing, extending to approximately an inch from the pulmonary periphery

and toward the dependent portions of the lung. The density has a "ground-glass" appearance and may become circumscribed. No increase in hilar adenopathy, no atelectasis or pleural reaction are usually noted. Serial roentgenograms usually show no change, which is an important factor in diagnosis. There is no lymphadenopathy.

A great aid in diagnosis of lipoid pneumonia is the study of the sputum for oil globules, as is also a lung biopsy if indicated. Precautions, however, should be taken not to induce a pneumothorax when a biopsy is done. It is important to differentiate the disease from pulmonary neoplasm in unilateral cases, and from pneumoconiosis, chronic bronchitis, and chronic pulmonary tuberculosis particularly in its disseminated hematogenous form.

There is no known method of treatment of lipoid pneumonia. Neither partial drainage nor therapeutic bronchoscopy is of value in a well-established disease. Rossier (5) suggests injection of epinephrine since he has found that the vital capacity is increased by this medication, probably because the dyspnea was partly due to spastic reaction of the bronchi to the foreign-body stimulus.

As a prophylactic measure, oil medication should not be administered before retiring since the material may trickle down into the trachea and bronchi during sleep. Oil should not be prescribed for a patient with a defective deglutition reflex. Precautionary measures should be taken in industry for people who are engaged in work with nebulized oil material.

The disease is usually chronic and lasts for months. The initial damage is not too great and, if no more oil gets into the lungs, recovery may slowly take place in uncomplicated cases. Otherwise, chronic fibrous abscesses with cavitation often result. Death may occur from secondary invasion of the lung by various bacteria or from exhaustion and asphyxiation by obliteration of normal tissue.

SUMMARY

Two cases of oil pneumonitis, one due to aspiration of nebulized industrial oil and the other due to the aspiration of fatty material because of difficulty in deglutition, are described.

SUMARIO

Neumonía Lipoidea

De los 2 casos de neumonitis oleosa aquí descritos, uno se debía a aspiración de aceite industrial nebulizado y el otro a aspiración de materias grasas por existir disfagia.

RESUME

Pneumonie Huileuse

Description de deux cas de pneumonie huileuse, l'une étant due à l'aspiration de huile industrielle nébulisée, l'autre par trouble de la déglutition.

REFERENCES

(1) Laughlen, G. F.: Studies on pneumonia following injections of oil, Am. J. Path., 1925, *1*, 407.

(2) Pinkerton, H.: Reaction to oils and fats in lung, Arch. Path., 1928, *5*, 380.

(3) Cannon, P. R.: The problem of lipid pneumonia, J.A.M.A., 1940, *115*, 2176.

(4) Moel, M., and Taylor, H. K.: Oil aspiration pneumonia, Am. J. Roentgenol., 1943, *49*, 177.

(5) Rossier, P. H., and Buhlmann, A.: Eine oelpneumonie nach Jahrenlangen Gebrauch Von flussigen Parafin Als Nasentropfen, Schweiz. med. Wchnschr., 1949, *79*, 685.

(6) Shashkas, M., Baufield, W. A., and Rosenbaum, S. I.: Distribution effect and fate of oil aerosol particles retained in the lungs of mice, Arch. Indust. Hyg. & Occup. Med., 1950, *1*, 20.

Notes

THE SPECTROPHOTOMETRIC DETERMINATION OF PARA-AMINOSALICYLIC ACID AND ITS ACETYL DERIVATIVE IN HUMAN URINE

In view of the desirability of a simple and specific method for the determination of para-aminosalicylic acid (PAS) and its metabolites, experiments were conducted to develop a spectrophotometric assay for these entities.

Preliminary studies of urine samples from patients receiving PAS were made using paper chromatography in an 8:1:1 butanol-water-acetic acid system, PAS and acetyl-para-aminosalicylic acid (AcPAS) being employed for comparison. After development, the components were located by fluorescence under ultraviolet light. These studies, together with spectrophotometric measurement of solvent partition in water-amyl acetate systems, disclosed the following:

(a) Following administration of PAS, the major metabolic product in human urine was AcPAS, which appeared together with unchanged PAS.

(b) PAS and AcPAS were the sole detectable components in amyl-acetate extracts of acidified metabolite urines.

(c) PAS could be partitioned into amyl acetate from 0.1 N HCl to yield constant and reproducible fractional recoveries in the solvent phase. Three washes with equal volumes of 1.0 N HCl completely removed PAS from the amyl acetate.

(d) AcPAS, under the same conditions, was quantitatively retained in the solvent phase.

(e) Amyl acetate extracts of acidified normal urine when washed three times with equal volumes of 0.1 N HCl exhibited no interfering absorption at λ 300 mu.

(f) Para-(di-*n*-propylsulfamyl)-benzoic acid (Benemid), a drug occasionally employed together with PAS in antituberculous therapy, exhibited no interfering absorption at λ 300 mu at concentrations normally found in urine.

Based on these findings, the following method was developed:

One milliliter of urine diluted with 9 ml. of 0.1 N HCl was extracted with 10 ml. of amyl acetate in a 50 ml. separatory funnel. The aqueous phase was discarded, and the solvent layer washed three times with 10 ml. portions of 0.1 N HCl.

One milliliter of the amyl acetate was withdrawn and diluted to 100 ml. with ethanol (Solution I).

The remaining solvent phase then was further washed with four 9 ml. portions of 1.0 N HCl. One milliliter was withdrawn and diluted as before (Solution II).

The absorption of the two solutions was read at λ 300 mu in a Beckman model D. U. spectrophotometer using silica cells at a slit width of 0.3 mm. The blank was amyl acetate washed with 1.0 N HCl and diluted 1 ml. to 100 ml. with ethanol.

Calculation of results: When run through the procedure, the mean values of PAS and AcPAS in aqueous solution or when added to normal urine were found to be:

	Density Solution I (D_I)	*Density Solution II* (D_{II})
1.00 mg. PAS per ml.	0.059	0.000
1.00 mg. PAS as AcPAS per ml.	0.053	0.051

From this it follows that:

$$\text{mg. PAS as AcPAS per ml. urine} = \frac{D_{II}}{0.051}\ (A),$$

$$\text{and: } D_I = 0.059 \times \text{mg. PAS per ml. urine} + \frac{0.053}{0.059}$$

$$\text{thus: mg. PAS per ml. urine} = \frac{D_I - 1.04\,D_{II}}{0.059}\ (B).$$

The use of equations A and B, therefore, permits the simultaneous determination of PAS and AcPAS (expressed as mg. equivalents of PAS). The application of this method to recoveries of the two compounds added to normal urine with or without Benemid is shown in table 1. The average deviation from the true values of all results in table 1 is 1.6 per cent, with the largest single error being 6 per cent (mg. PAS found, line 3).

TABLE 1

Recoveries of PAS and AcPAS from Urine, With and Without Benemid

	D_I*	D_{II}†	PAS ADDED (mg. per ml.)	AcPAS ADDED (mg. per ml.)	PAS FOUND (mg. per ml.)	AcPAS FOUND (mg. per ml.)
Normal urine	0.237	0.002	4.00	0	3.98	0.04
Normal urine	0.212	0.204	0	4.00	0	4.00
Normal urine	0.232	0.103	2.00	2.00	2.12	2.02
Normal urine plus 1 mg. per ml. Benemid	0.237	0.003	4.00	0	3.97	0.06
Normal urine plus 1 mg. per ml. Benemid	0.216	0.204	0	4.00	0.07	4.00
Normal urine plus 1 mg. per ml. Benemid	0.225	0.105	2.00	2.00	1.97	2.06

* D_I is the optical density, determined spectrophotometrically at λ 300 mu, of Solution I.
† D_{II} is the optical density, similarly determined, of Solution II (see text).

It is possible that occasional highly pigmented urines may exhibit a greater interference.

Two precautions may be mentioned in connection with this determination. Obviously the method cannot be applied to urines of patients receiving other drugs which give rise to substances absorbing at λ 300 mu; acetylsalicylic acid and sodium salicylate are examples. Furthermore, we have noticed that chilled or frozen samples may deposit crystals of AcPAS. These must be redissolved by gentle heating if valid results are to be obtained.

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July 17, 1951

NOTES

The following communication from the Division of Chronic Disease and Tuberculosis of the Public Health Service and accompanying "Report and Recommendations of the Committee on Tuberculosis Morbidity Reporting," which was presented by the Committee at a joint meeting of State Tuberculosis Control Officers and State Sanatoria Directors, held in connection with the annual meeting of the National Tuberculosis Association in Cincinnati, May 14, 1951 and accepted by the members of the joint meeting at that time, are reprinted from *Public Health Reports*, October 1951, for the benefit of readers of THE AMERICAN REVIEW OF TUBERCULOSIS. The subject has been under consideration repeatedly in joint meetings of the staff of the Division of Tuberculosis (now merged in the Division of Chronic Disease and Tuberculosis) and the staff of the National Tuberculosis Association. Members of the Association and its medical section, the American Trudeau Society, will welcome the appearance of the Report in printed form.

WHAT IS A REPORTABLE CASE OF TUBERCULOSIS?

The reporting of cases is indispensable to the control of tuberculosis. A health department cannot discharge its responsibilities for the supervision of cases and the examination of contacts if all cases identified in its jurisdiction are not brought promptly to its attention. The case that is reported first on a death certificate represents to the tuberculosis controller a disheartening frustration of his aims.

Furthermore, reliable information about the extent of the problem is necessary for intelligent planning of control programs. In the thirty years of almost universal compulsory reporting of tuberculosis in this country, there has been so little agreement on definitions of reportability or uniformity in reporting practices that available tuberculosis morbidity data have represented only makeshift estimates. Mortality rates have been used as a measure of control needs, in spite of growing awareness of their inadequacy for that purpose. In recent years, the rapid decline in the death rate has quite evidently not been accompanied by a commensurate decline in morbidity. As case-finding efforts are carried on with increasing vigor and more cases are discovered in the minimal stage, and as new drugs and other methods of therapy prolong the lives of tuberculosis patients, it is apparent that mortality rates are no longer an accurate current guide to the tuberculosis problem. There is greater need for better reporting of all cases.

To be really useful, moreover, tuberculosis case reporting should conform to commonly accepted standards. Data are of little use if some reflect all cases including suspects, while others show only active cases with positive bacterial findings.

Recognizing that the epidemiology of tuberculosis would continue to be based largely upon presumptions and not upon facts until case reporting was standardized, the Joint Meeting of the State Tuberculosis Control Officers and State Sanatoria Directors in 1949 recommended the appointment of a committee to study the problems of tuberculosis morbidity reporting and to try to work out solutions. Members of the Committee appointed by Dr. R. J. Anderson, Chief, Division of Tuberculosis, Public Health Service, represent states in widely separated parts of the country, with various population characteristics and tuberculosis control patterns. They are:

Dr. C. M. Sharp
Director, Bureau of Tuberculosis,
Florida State Board of Health

Dr. Richard M. Burke
Director, Division of Tuberculosis Control
Oklahoma State Health Department

Dr. Edward X. Mikol
General Director of Tuberculosis Hospitals
Division of Tuberculosis Control
New York State Department of Health

Dr. Paul S. Phelps
Director, Connecticut State Tuberculosis Commission

Dr. Edward Kupka
Chief, Tuberculosis Service
California State Department of Public Health

Dr. Cedric Northrop
Head, Tuberculosis Control Section
Washington State Department of Health

Dr. Hilbert Mark[1]
Tuberculosis Control Officer
Division of Preventable Diseases
Minnesota Department of Health

This committee has met several times in the past two years and has considered in detail the ramifications of the assigned subject. They have dealt with exceedingly perplexing questions, and their final report, which was accepted by the Joint Meeting in May of 1951, is a valuable contribution to tuberculosis control.

The committee was not concerned with criteria for the diagnosis of tuberculosis. These are defined in DIAGNOSTIC STANDARDS AND CLASSIFICATION OF TUBERCULOSIS, published by the National Tuberculosis Association, a new edition of which was issued in 1950. The Committee on Tuberculosis Morbidity Reporting used this publication in its work but its problem was to define standards for the *reporting* of cases, not for diagnosis.

The report provides for standards for tuberculosis reporting which are sufficiently flexible to allow for the differing needs and regulations of the various states, and at the same time will produce comparable data. The recommendations of the Committee also provide for some local variation in the selection of reportable cases and in the mechanism of reporting. If they are followed, however, they will yield significant information which has heretofore been lacking.

The Tuberculosis Control Officers and State Sanatoria Directors, in accepting the Committee's recommendations, indicated their interest in improving the status of tuberculosis morbidity reporting. We then will have the means whereby the confusion which has accompanied tuberculosis case reporting can be eliminated. This is possible only if action is taken by all health jurisdictions to adopt the Committee's proposals. The ultimate effectiveness of the Committee's work will depend upon general application of the recommendations.

REPORT AND RECOMMENDATIONS OF THE COMMITTEE ON TUBERCULOSIS MORBIDITY REPORTING

May 14, 1951

I. Purposes of Reporting Tuberculosis Cases

The primary purposes of tuberculosis morbidity reporting are:

A. For case supervision—to assure continued medical supervision, isolation, and follow-up of all known cases as long as may be necessary to prevent spread of the disease

[1] Served on Committee from August 1949 to December 1950; at present, Director of the Division of Tuberculosis Control, Denver City Health Department.

B. For contact supervision—to facilitate the examination of contacts of all known cases
C. For program management—to provide information for use in:
   1. Determining the extent and characteristics of the tuberculosis problem, and
   2. Evaluating the effectiveness of tuberculosis control measures.

II. Medical Criteria for Reporting Tuberculosis Cases

A. *To be reported*
   The following cases should be reported:
   1. Cases with tubercle bacilli demonstrated
   2. Cases with other significant evidence, even though bacteriological proof has not yet been demonstrated, such as
      a. Chest X-ray shadows characteristic of active tuberculosis (soft infiltrate, cavity, et cetera).
      b. Unexplained pleurisy with effusion
      c. Clinically active extrapulmonary tuberculosis (meningeal, bone, kidney, et cetera).

B. *May be reported*
   The following previously unreported tuberculosis cases may be reported:
   1. Cases of pulmonary fibrosis and nodulation more than minimal in extent, presumably of tuberculous origin
   2. Cases with a record of active disease or previous treatment within the past 5 years
   3. Cases with X-ray evidence of collapse therapy or resection for tuberculosis
   4. Active primary pulmonary tuberculosis cases.

C. *Not to be reported*
   The presence of the following should not be considered sufficient evidence for reporting:
   1. Chest X-ray film revealing fibrous lesions, minimal in extent, without history or symptoms (This includes fine linear strands; discrete, hard nodules; and apical-pleural scars.)
   2. Positive tuberculin reaction only
   3. Pulmonary calcification, including healed primary tuberculosis
   4. Healed extrapulmonary tuberculosis.

III. Report Forms

A. *Methods of reporting*
   The Committee makes no specific recommendations as to the type of report form which should be used, except that each case should be reported on an individual form. It recognises that there are at least four satisfactory methods of reporting:
   1. General morbidity report form followed by a special tuberculosis morbidity report form
   2. Special morbidity report form for all cases of tuberculosis
   3. Special morbidity report form for initial use by certain sources of reports such as clinics and tuberculosis hospitals; other sources to use method in 1, above
   4. Combined general morbidity report form with a section for special tuberculosis information.

   The Committee feels that a general morbidity report without the minimum medical information indicated in C, below, is inadequate.

B. *Minimum identifying information*
   Name, address (present and usual, if different)
   Age, sex, and race
   Source of report
   Date reported
   Was case first found on miniature X-ray?

C. *Minimum medical information*

Form and extent	*Activity status*	*Bacterial status*
Pulmonary	Active*	Positive
Minimal		
Moderately advanced	Activity undetermined	Negative
Far advanced	Probably active*	
Other, specify ——	Probably inactive	Unknown
Non-pulmonary, specify ——	Arrested	
	With positive bacterial findings*	
	With negative bacterial findings	
	Inactive	

D. *Optional information*

Occupation
Marital status
Veteran status
Length of state residence
Date of last laboratory examination
Date of last X-ray examination
If reported after death, date of death
Was diagnosis verified by X-ray, laboratory examination, or autopsy?
Method of laboratory examination (direct smear, concentrated smear, culture, animal inoculation)
Who is to supervise the case?

IV. Administrative Procedures

A. A newly reported tuberculosis case in any state is one which, in addition to meeting the medical criteria presented above, has never been previously reported to the state.

B. There should be a reasonable amount of screening and querying for the purpose of improving reporting and the comparability of data.

C. Tuberculosis cases are sometimes first reported to health departments by Federal, state or local agencies, or physicians by telephone, letter, or forms other than the official morbidity report (Veterans Administration form, clinic examination reports, death certificates, interstate report notices, et cetera). Information on such cases should be entered on the official morbidity report form so that they may be counted as new cases.

D. The following are the recommended procedures for counting cases on an annual basis:

1. A case is counted in the year in which it is reported, provided that the report is received by the state health department by January 31 of the following year. If received after that date, it is counted in the year in which it is received.
2. When follow-up information which reverses the diagnosis or otherwise provides a basis for not counting a case is received during the year the case was reported or during January of the following year, the case is deleted from the count. However, if such information is received after January 31 no change is made in tabulations of newly reported tuberculosis cases.

E. Active and probably active tuberculosis cases, as defined in Section II. A., shall always be tabulated as a group and separately from the cases in the optional categories of Section II. B. Tabulations made by stage of disease should also follow this grouping of activity status, as should tabulations for other characteristics such as age, sex, and race. In this way a reasonable degree of comparability between states will be achieved.

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* Cases in these classifications shall always be reported. Whether or not cases in the other classifications of activity status are reported will be determined by state policy.

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ABSTRACTS

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ABSTRACTS

CLINICAL STUDIES, TUBERCULOSIS

ACTH and Tuberculosis.—The effect of adrenocorticotropic hormone (ACTH) and cortisone on the course of tuberculosis was studied in 7 patients. Abrupt and profound symptomatic improvement resulted in both febrile and afebrile patients. Although little change occurred in the roentgenographic appearance, laryngeal lesions cleared remarkably as noted by direct visualization, and in some cases there was a reversal of cutaneous hypersensitivity to tuberculin. With cessation of therapy all signs and symptoms reverted to the pretreatment stage or became worse; and, in addition, titers of tuberculin hemagglutinating antibodies and serum gamma globulin level rose. In 2 patients, despite prolonged therapy, the course was downhill with relapse of symptoms and subsequent death.—*Effects of adrenocorticotropic hormone and cortisone in patients with tuberculosis, C. LeMaistre, R. Tompsett, C. Muschenheim, J. Moore, & W. McDermott, J. Clin. Investigation, May, 1951, 30: 445—(S. Hadley)*

Hyponatremia in Tuberculosis.—Ten patients with low serum sodium levels and far advanced pulmonary tuberculosis were studied. All were markedly malnourished with hypoalbuminemia but none showed evidence of renal or adrenal disease. When excess salt was given in the diet, the urine sodium remained high despite blood levels of 122 to 131 mEq. per liter, and on very low salt intake the sodium excretions were also above normal. None of the usual clinical signs of sodium depletion were manifest. There was no definite evidence of response to adrenal cortical extract but increased quantities of sodium were retained when desoxycorticosterone was administered. The syndrome can be differentiated from Addison's disease as an eosinophil response to epinephrine occurs and there is an ability to reduce sodium excretion on a dietary regimen free of salt. The authors postulate that a primary reduction in cellular osmolarity in the syndrome may be the initiating factor. This results in an increased excretion of sodium in an attempt to reduce extracellular tonicity to conform with the reduced cellular osmolarity.—*Asymptomatic hyponatremia in pulmonary tuberculosis, E. Sims, L. Welt, J. Orloff, & J. Needham, J. Clin. Investigation, December, 1950, 29: 1545.—(S. Hadley)*

"Positive Pressure" Roentgenograms.—Occasionally surprising differences in the size of the heart and in the filling of the vessels may be found in consecutive roentgenograms taken apparently with the same technique. These variations may be ascribed to the manner in which patients "take a deep breath and hold it." During forced expiration against a closed glottis, the intra-alveolar pressure rises, the pulmonary vessels are compressed, and less blood is returned to the right heart. In consequence, the cardiac output is reduced, the pulmonary vessels fill less completely, and eventually, less blood returns to the left heart. Westermark has developed a technique for taking chest roentgenograms while the intra-alveolar pressures are maintained at plus 40 cm. to minus 40 cm. of water. The diagnostic value of this method was studied in diseases of the heart and lungs. The technique was found to be of real value in cases of pulmonary congestion

and edema, of acute pneumonic changes, for a better visualization of hilar nodes, and for early diagnosis of mitral valve disease. No striking changes were found in emphysema, atelectasis, chronic inflammatory processes, or in pulmonary tuberculosis, the tuberculous lesions remaining essentially unchanged. In only one case of fibrocaseous disease was a slight decrease in size of the lesion possibly due to the decrease in the perifocal hyperemia. In 10 cases the lesions were slightly better visualized and appeared more sharply defined by the "wiping out" of the vessel markings in the surrounding lung tissue. In one case the lesion appeared larger on the positive pressure roentgenogram. In 2 patients with fine nodular disseminated disease, the lesions were better visualized in the positive pressure films as the diffuse vascular pattern became less prominent.—*The limited usefulness of the "positive pressure" chest roentgenogram in pulmonary tuberculosis, B. Pollak & A. Hurst, Am. J. Roentgenol., June, 1951, 65: 886.—(T. H. Noehren)*

Tuberculoma and Inspissated Cavity.—Round, well-circumscribed pulmonary lesions are often diagnosed tuberculomas when they are in reality inspissated cavities or the fibrous end-stage of cavities. Tuberculomas should be diagnosed only when one knows the pathogenesis of the round focus although in the future the therapy of tuberculoma as well as of inspissated cavity will be partial resection. Pathologically, the tuberculoma is a localized fibrocaseous pneumonia often arranged in concentric layers because the enlargement of the lesion occurs concentrically. Microscopically, the elastic network of the alveoli is visible even within the caseous material. The inspissated cavity is a caseous mass usually without concentric layers and with no evidence of elastic alveolar structure. In discussing this paper, Fourestier reported a roentgenologic sign for differential diagnosis. Careful tomographic studies show in the case of an inspissated cavity the presence of a small bronchial image leading towards the round focus; in the case of a tuberculoma, no evidence of a draining bronchus is present. —*Contribution au diagnostic différentiel des tuberculomes du poumon, R. B. Spanjaard, Rev. de la tuberc., 1951, 15: 133.*—(*V. Leites*)

Bronchography in Tuberculosis.—Bronchography has not been used extensively in tuberculosis because of the fear that the disease may spread as the oil is coughed up, because residual oil might interfere with the interpretation of subsequent chest films, and because of the fear that iodine may harm the patient. In a study of 100 patients, none of these untoward effects was noted. The cases studied were those in which the medical or surgical therapy seemed adequate and bronchoscopy was negative but positive sputum persisted. Special efforts were made to fill the particular segmental bronchi by posturing, this procedure demonstrating that most of the lesions were found in the dorsal branches of the upper and lower lobes. The decision to perform a resection rather than a thoracoplasty was made in several cases as the result of demonstrating bronchiectatic dilatations or occlusion of a segmental branch. Of the 100 bronchograms done, 12 were normal, 83 abnormal, and 5 unsatisfactory fillings.—*Bronchography in pulmonary tuberculosis, P. Rabinowitz & I. S. H. Harper, Dis. of Chest, January, 1951, 19: 58.*—(*E. A. Rouff*)

Pneumonolysis Complications.—Over a three-year period, 132 pneumonolyses were performed on 110 patients. Evaluation six months postoperatively revealed that in only 60 patients was a satisfactory pneumothorax maintained, 3 others had an unexpandable lung with pneumothorax, and in 33 the collapse had been abandoned. At the time of the lysis, 40 per cent of the operations were considered to be completely successful and 44 per cent only partially complete. In 56 cases an appreciable amount of serous fluid developed postoperatively; in 8, tuberculous empyema appeared; and in 3, mixed and pyogenic empyema. Obliterative pleuritis developed in 39 cases, and in 27 of these

there was associated serous fluid.—*Complications of pneumothorax and pneumonolysis, L. H. Stug & R. M. Shepard, Dis. of Chest, January, 1951, 19: 78.—(E. A. Rouff)*

Surgery for Tuberculous Empyema.—The results of pleural pneumonectomy for tuberculous empyema were compared with the results of the "classical" methods of treatment. Between 1940 and 1948, 192 patients with severe tuberculous empyema were treated with thoracotomy, aspiration, thoracoplasty, or pleurectomy; 20 per cent recovered completely. There was a partial result in 20 per cent with persistence of a residual pleural space. The over-all mortality was 60 per cent. Of the 192 patients, 15 died before thoracotomy could be performed, 177 had thoracotomy. In only 9 of these could a durable result be obtained by aspiration alone; 86 patients in whom a thoracoplasty could not be performed died after a variable period of illness. Thus, patients treated with thoracotomy alone had a mortality of 80 per cent, 10 per cent of cures, and 10 per cent of persistent fistulas. Patients treated with thoracotomy and thoracoplasty had a mortality of 15 per cent, 50 per cent of cures, and 35 per cent of persistent fistulas. During the last three years 30 patients with severe tuberculous empyema were treated with pleural pneumonectomy. The operation was successful in 6 of 8 cases of empyema and fistula associated with extrapleural pneumothorax; 22 patients had empyema as a consequence of intrapleural pneumothorax, 12 having a bronchopleural fistula. In all cases active lesions were found in the underlying lung. The mortality was slightly more than 20 per cent; the operation was considered successful in almost 80 per cent. Thoracoplasty subsequent to resection was performed in 9 of 17 cases. For patients with tuberculous empyema who have not undergone thoracotomy and who have unilateral tuberculosis, pleural pneumonectomy without preceding drainage is the treatment of choice. The indication is the more imperative the more recent the pleural complication, the more extensive the pulmonary lesion, and the larger the bronchopleural fistula. The presence of bronchial stenosis further favors resection. The general condition of the patient is of secondary importance and one can intervene even in highly febrile patients. The elimination of the active lesions is followed by a remarkably rapid diminution of constitutional symptoms. Early resection increases the rapidity of the success and the quality of the end result. In ases of hronic empyema with tube drainage and well-stabilized pulmonary lesions, radical intervention would have to be undertaken under technically more difficult conditions. The resistance of these patients is decreased by the long-standing purulent process and the presence of amyloidosis. A thoracoplasty is preferable in these cases; it can be followed by a pleural pneumonectomy at a later date if necessary.—*Indications actuelles dans le traitement chirurgical des pleurésies purulentes tuberculeuses, M. Berard, M. Jaubert de Beaujeu, & P. Justin, Rev. de la tuberc., 1951, 15: 118.—(V. Leites)*

Surgery for Tuberculous Empyema.—A total of 277 thoracoplasties were performed for long-standing tuberculous empyema with bronchopleural fistula, with only 28.1 per cent of recoveries. In 132 cases, pleurectomy had to be resorted to; 80.8 per cent of these showed great improvement, 72 per cent were completely cured locally, 64.8 per cent obtained a more durable result. Five of the "cured" patients and 3 others who are greatly improved now have a soft axillary chest wall. A pleural pneumonectomy performed earlier would have been preferable in all of these patients who also had extensive pulmonary lesions. Decortication could not have been proposed to most of them because of the severe involvement of lung and visceral pleura (Authors' summary).—*Indications relatives des différentes techniques chirurgicales dans le traitement des pyothorax tuberculeux, A. Bernou, R. Goyer, L. Marécaux, & J. Tricoire, Rev. de la tuberc., 1951, 15: 120.—(V. Leites)*

Bronchial Fistula.—Bronchial fistula from erosion of a caseous node was found bronchoscopically in 13 per cent of 247 cases of active primary tuberculosis in children. Since many bronchial fistulas are invisible during bronchoscopy and since, for technical reasons, bronchoscopy was not performed as frequently as desired, this figure probably is lower than the real incidence. Cases of lobar and segmental consolidation showed fistula formation in 39 per cent. It was not unusual to observe development of fistulas late in the course of primary tuberculosis. Discrete pulmonary infiltrations in the adjacent area were found in 22 per cent of the cases. The duration of the fistulas was from one month to more than a year. The outcome was good in all cases; there were no deaths and no progressive "primary phthisis." Fistula formation is the sign of a locally severe adenopathy, and it was frequently associated with irreversible changes in the lung parenchyma such as atelectasis and bronchiectasis.—*La fistulisation ganglionnaire au cours de la primo-infection tuberculeuse de l'enfant (à propos de 41 observations personelles), M. Jeune, P. Mounier-Kuhn, & F. Potton, Semaine d. hôp. Paris, May 2, 1951, 27: 1428.*—(*V. Leites*)

CLINICAL STUDIES, NONTUBERCULOUS DISEASES

Pregnancy in Sarcoidosis.—Three patients with sarcoidosis were followed during and after pregnancy. All 3 had pulmonary involvement; 2 had generalized lymph node disease and the third had involvement of the skin and bones. Case 1 experienced an asymptomatic exacerbation of her pulmonary disease following delivery. Her chest cleared considerably within eight months and has remained essentially the same. The baby was normal, and she has since delivered another normal child with no ill effects. The pregnancies and postpartum courses were completely uneventful in Cases 2 and 3. Normal babies were delivered, and there was no demonstrable change in the course of the disease. Case 2 remained well for three years, when she was again seen for possible kidney involvement but it is doubtful that this could be related to her pregnancy. Case 3 was followed for one and a half years after delivery and steadily improved during this time. It is believed that sarcoidosis is not a contraindication to pregnancy and that pregnancy does not significantly alter the course of the disease.—*Sarcoidosis: V. The effects of pregnancy on the course of the disease, S. W. Donaldson, A. C. Tompsett, R. H. Grekin, & A. C. Curtis, Ann. Int. Med., May, 1951, 34: 1213.*—(*T. H. Noehren*)

Fibrinolysins for Hemothorax.—Occasionally blood clots in the pleural space before it can be aspirated. Surgery offers decortication for the uninfected case; and decortication, tube drainage, and the Schede operation for empyemas. Streptokinase (SK) and streptodornase (SD) are streptococcal products which, with the antibiotics, may decrease the need for such surgery. They help dissolve clots and thin pus by catalytic and enzymatic action. A fistula is not produced but it may be made apparent by the lytic action. A known fistula and a well-organized fibrous peel are the only contraindications. In 10 cases, SK and SD were used for a diversity of indications. The optimal time for treatment seemed to be fourteen days after hemorrhage; the optimal interval between instillations, approximately two days. The usual preparation contained 200,000 units of SK and 70,000 units of SD in 30 cc. of physiological saline solution; 500,000 units of penicillin and 1 gm. of streptomycin were also instilled in infected cases unless bacterial studies indicated some other antibiotic. Fever, malaise, and pleural pain were decreased by the use of amidopyrine. In the present series there were 3 cases of clotted traumatic hemothorax, 2 cases of clotted extrapleural pneumothorax, and one case each of hemothorax after segmental resection, fibrin ball in extrapleular pneumothorax, clotted infected hemothorax, post-pneumonectomy empyema, and tuberculous empyema. The results were encouraging.—*Streptokinase and antibiotics in the*

treatment of clotted hemothorax, D. Carr & S. G. Robbins, Ann. Surg., June, 1951, 133: 853.—(W. H. Oatway, Jr.)

Pulmonary Fibrosis of Unknown Origin.—A 42-year-old patient died of bilateral pulmonary fibrosis of unknown etiology. The disease process, which was followed both clinically and roentgenologically for four years, extended in caudo-cranial direction and developed earlier on the right side than on the left. Post-mortem pathologic examination showed diffuse, bilateral, chronic, interstitial pneumonia of all lobes with extensive metaplasia of the bronchial epithelium, formation of many giant cells, and hyperplasia of smooth muscle. The proliferative inflammatory process decreased from bottom to top of each lung, was more developed on the right than on the left, was most advanced peribronchially, and was accompanied by bronchiolar ectasia. It is possible that the noxa of this infection was an unknown epitheliotropic virus.—*Een geval van fibrosis pulmonum e causa ignota, J. F. Ph. Hers, Nederl. tijdschr. v. geneesk., May 19, 1951, 95: 1495.—(J. van der Vate).*

Lipiodol and Pulmonary Function.—These studies were intended to clarify the need for delay in operative procedures following lipiodol studies. Determinations were made in 10 patients of alveolar-arterial oxygen pressure gradients, arterial oxygen saturation, and vital capacity before and after lipiodol. In only one patient was the alveolar-arterial oxygen pressure gradient definitely decreased after lipiodol although 5 cases in addition to this one had abnormally high gradients in the control study. The arterial carbon dioxide tension was essentially unchanged. Vital capacity was reduced 7.6 per cent after bronchography. There was no significant effect on the time required for saturation of arterial blood on changing the inspired gas from room air to 100 per cent oxygen. Any abnormal values returned to normal levels within twenty-four hours with one exception, in which case forty-eight hours were required. It is concluded that the presence of lipiodol has no significant influence on the respiratory gas exchange.—*The effect of lipiodol on alveolar gas exchange, H. Black & A. Roos, J. Clin. Investigation, March, 1951, 30: 338.—(S. Hadley)*

Pulmonary Autonomic System.—Pulmonary arteriolar resistance was measured in 15 subjects, using the Hellems technique. Studies were made before and after the administration of tetraethylammonium chloride. Results showed a significant fall in pulmonary arteriolar pressure in 11 of 15 subjects. In the 4 normal patients, there was no fall in pulmonary arteriolar resistance. In 4 of 6 cases of pulmonary hypertension, there was a significant decrease in pressure. This suggests that at least one component in pulmonary hypertension is mediated through the autonomic nervous system.—*Observations on autonomic participation in pulmonary arteriolar resistance in man, N. Fowler, R. Westcott, V. Hauenstein, R. Scott, & J. McQuire, J. Clin. Investigation, October, 1950, 29: 1387.—(S. Hadley)*

Morphine in Cor Pulmonale.—A patient with chronic bronchitis and emphysema complicated by infection was given morphine. He lapsed into coma and died. This phenomenon has been noted previously. The usual explanation which is invoked is that cough is depressed and secretions stagnate. There is a much more important factor, however, which contributes to the unfavorable outcome. It is essential that the respiratory center remain sensitive to decreased oxygen tension of the blood so that there may be maximal respiratory effort to overcome the effects of the greatly diminished vital capacity. Morphine decreases or abolishes this sensitivity and anoxia ensues. The untoward effect of the drug, if detected early enough, can be counteracted by the slow intravenous administration of 5 to 10 cc. of nikethamide and by oxygen therapy. Whereas morphine is life saving in counteracting the useless dyspnea of other forms of cardiac failure, it may be lethal when used to counteract the compensa-

tory hyperpnea of cor pulmonale.—*Lethal effect of morphine in chronic cor pulmonale, N. J. Roussak, Lancet, May 26, 1951, 1: 1156.*—(*A. G. Cohen*)

Leiomyoma of the Esophagus.—Leiomyomata comprise 75 per cent of the benign tumors of the esophagus and are found in 0.4 per cent of autopsies. The removal of such tumors surgically has been reported in 14 cases. Two instances of treatment by simple enucleation are discussed in this paper. The first patient, a young man, was seen about a year before operation with a two-year history of vague distress at night, a feeling of difficulty in breathing, some dysphagia, occasional eructations, and substernal pain. Roentgenography showed a large rounded tumor above the arch of the aorta. Barium in the esophageal lumen demonstrated a moderate dilatation above the lower border of the second rib and considerable narrowing below it. The tumor lay between the trachea and the esophagus. At operation the esophagus was opened for three or four centimeters above the tumor which was found to arise in the esophageal wall between the muscularis and the mucosa. The mucosa was sutured and the mass, resembling a uterine fibroma, was removed by enucleation. Histologically, it was a benign leiomyoma. In the second case, the symptoms and findings were similar. A diagnosis of benign leiomyoma was made before operation and the tumor was removed by enucleation without previously opening the esophagus. Histologic examination confirmed the diagnosis. Two procedures are possible in cases of progressive, symptomatic leiomyoma of the esophagus: (*1*) resection of the esophagus and (*2*) enucleation. The latter is much simpler and is preferred in all cases where the integrity of the mucosa can be preserved. When cleavage cannot be accomplished without serious risk of injuring the mucosa, resection becomes necessary.—*Deux cas de léiomyome de l'oesophage traités par énucléation simple, Chalnot, Faivre, & Lechard, Poumon, January, 1951, 7: 19.*—(*A. T. Laird*)

Pulmonary Resection in Children.—Resectional surgery in children presents special features. Obstruction of the tracheobronchial tree with its complications, anoxia, and atelectasis have to be more seriously considered than in the adult because of the small dimensions of the airways. Replacement of blood loss has to be more exact than in the adult because of the low circulatory reserve and the danger of overloading the circulation. On the other hand, recuperation in children is more rapid and more complete than in the adult. Of 44 pulmonary resections in children, 9 were pneumonectomies and 35 partial resections for bronchiectasis (20 cases), abscess or pulmonary suppuration (10 cases), tuberculosis (3 cases), emphysematous bulla and similar conditions (5 cases), pulmonary cysts (4 cases), infected echinococcus cyst (one case). There were 4 deaths. In the remaining 40 cases, the immediate postoperative complications were transient atelectasis (13 cases) and empyema (one case). The latter responded well to aspirations and antibiotics. The long-term results were on the whole very favorable. Respiratory function was satisfactory, permitting the children to lead a normal life (Author's summary).—*Les résections pulmonaires chez l'enfant, J. Mathey, Semaine d. hôp. Paris, April 26, 1951, 27: 1359.*—(*V. Leites*)

Pleural Endometriosis.—A case was observed in which there was a recurrent massive, sanguineous right pleural effusion. At thoracotomy, the surface of the lung was found to be covered by a thin pellicle. Most of this was removed. Histologic examination showed typical endometrium. A gynecologic investigation did not show evidence of endometriosis. Testosterone tablets were implanted subcutaneously. Fourteen months later there was a recurrence of the effusion. Oophorectomy was carried out and endometrial deposits were seen on the pelvic peritoneum.—*Endometriosis of the pleura, H. Nicholson, Thorax, March, 1951, 6: 75.*—(*A. G. Cohen*)—

Angiocardiography in Carcinoma.—In angiocardiograms of 5 cases of bronchogenic carcinoma, the main pulmonary artery of the involved lung appeared "amputated." The functional pulmonary blood supply was completely abolished in this lung because the pulmonary artery was compressed by the tumor or by metastatic lymph nodes. Diagnostically, this angiocardiographic finding indicates the probability of a malignant process. Prognostically, amputation of the pulmonary artery implies that resection will be difficult or impossible. Exploratory thoracotomy remains, however, of prime importance and surgical exploration of the mediastinum often demonstrates the possibility of resection. Errors can be made if angiocardiography is used alone. In the presence of an amputated artery, circulatory and respiratory compensation takes place progressively in the contralateral lung. Pneumonectomy, therefore, will not be followed by functional difficulties in the immediate postoperative period, in contrast to cases where a well-functioning lung has been resected.—*Le poumon exclu (syndrome d'amputation de l'artère pulmonaire a l'angiopneumographie), M. Barilty, O. Monod, P. Choubrac, & P. Joly, Presse méd., May 23, 1951: 711.*—(*V. Leites*)

Traumatic Chylothorax.—Two cases are reported of very young children on whom operation for congenital heart disease had been performed. Toward the end of the second week, chylothorax appeared. The patients were reoperated upon. Chyle was seen to be coming from a rent in the mediastinal pleura. Although the duct was not identified, the region was transfixed with sutures. The chylothorax disappeared.—*Surgical management of traumatic chylothorax, H. B. Shumacker, Jr. & T. C. Moore, Surg., Gynec. & Obst., July, 1951, 93: 46.*—(*A. G. Cohen*)

Myasthenia Gravis After Thymectomy.—A patient with a malignant tumor of the thymus gland presented the first symptom of myasthenia gravis postoperatively. There was massive involvement of the cardiac musculature by the tumor but the patient lived fourteen months after the appearance of symptoms. Intensive roentgenotherapy may have contributed to his partial recovery and have prolonged his life materially.—*Malignant tumor of the thymus gland, myasthenia gravis developing after removal, J. B. Fershland & R. R. Shaw, Ann. Int. Med., April, 1951, 34: 1025.*—(*T. H. Noehren*)

Kartagener's Syndrome.—Kartagener's triad, a syndrome consisting of situs inversus, bronchiectasis, and sinus disease was found in a 49-year-old white man. There is a high incidence of bronchiectasis with dextrocardia but the reason for it is uncertain. In patients without upper respiratory infection or bronchial obstruction, congenital defects in the bronchial wall may be factors in the development of bronchiectasis.—*Kartagener's syndrome, G. M. Murray, Canad. M. A. J., March, 1951, 64: 248.*—(*E. A. Riley*)

ACTH in Beryllium Poisoning.—The therapeutic effects of ACTH and cortisone were evaluated in 22 cases of chronic beryllium poisoning; all but 3 patients received hormone therapy. Improvement, mostly temporary, was noted in all but 2 treated cases and was slight in another 2 patients. One of the untreated cases showed a spontaneous remission following an acute gastroenteritis. In the other, complete and spontaneous remission followed lobar pneumonia in a patient who was previously bedridden. In some cases, beryllosis regressed with discontinuance of the drug, and a second therapeutic course was not as uniformly successful as the first. The hormone treatment in one case studied histologically caused the epithelioid cells to disappear from the granulomas. Physiologically, the hormone produced: (1) hemodilution, (2) gain of weight, (3) a transient increase of maximum breathing capacity, (4) a slight increase of total lung volume, (5) an elevation of arterial blood oxygen partial pressure and a reduction of alveolar-arterial oxygen difference, (6) an increase in arterial

hemoglobin oxygen saturation, and (7) a reduction of the oxygen ventilation equivalent. It is suggested that, since the primary physiologic defect in beryllium granulomatosis is interference with the diffusion of oxygen, measurements of the efficacy of therapy should include physiologic studies of this defect. Agreement was not reached as to whether hormone therapy should be the drug of choice in this disease or whether it should be reserved for the more seriously affected patients.—*Symposium on the treatment of chronic beryllium poisoning with ACTH and cortisone, J. M. De Nardi, R. J. Hasterlik, E. M. Kline, S. R. Inkley, W. H. Pritchard, O. A. Sander, J. W. Cass, Jr., G. K. Fenn, P. W. Reynolds, H. L. Hardy, F. C. Bartter, A. E. Jaffin, H. S. Van Ordstrand, T. F. Frawley, J. McClement, B. G. Ferris, Jr., J. E. Affeldt, H. A. Kriete, J. L. Whittenberger, G. W. Wright, A. Forbes, F. Klemperer, Arch. Indust. Hyg. & Occup. Med., June, 1951, 3: 543–650.*—(*T. H. Noehren*)

ANTIMICROBIAL THERAPY

Meningitis After Streptomycin.—Tuberculous meningitis seems to have become more frequent since streptomycin has been widely used for the therapy of tuberculosis. At the Carlo Forlanini Institute in Rome the proportion of meningitis cases to the total number of hospitalized cases has increased from 0.77 per cent in 1937 and 0.43 per cent in 1940 to 1.60 per cent in 1948 and 2.09 per cent in 1949. There seems to be some evidence that this increase may be due to meningitis developing more often than normally expected n patients already treated with streptomycin. To clarify this point, the histories of 76 patients with tuberculous meningitis were studied. The first group of 38 had developed meningitis after streptomycin treatment; the second group of 38 had never before received streptomycin. The case distribution differed somewhat between the two groups. In the first group there were 2 cases of primary tuberculosis, 8 of acute and subacute miliary spread, 7 of chronic miliary disease, 17 of fibroulcerative tuberculosis under pneumothorax treatment, one case of nodular tuberculosis, and 3 of bone tuberculosis. In the second group there were 9 cases of clinically primary meningitis, 2 of primary tuberculosis, 7 of acute or subacute miliary spread, 9 of chronic miliary disease, 5 of fibroulcerative tuberculosis treated by pneumothorax, and 6 cases of bone tuberculosis. Meningeal complications were most often encountered in patients who had shown some improvement or had apparently reached clinical stabilization of their initial process. In the group of 38 previously treated patients 34 died and only 4 (11 per cent) recovered from the meningitis, notwithstanding renewed streptomycin therapy. In the other group, 19 of the 38 patients who had never been treated before with streptomycin are still alive. In the first group, 20 patients died within the first month and 9 more within the third month after onset of meningitis, while in the second group mortality was highest between the third and the twelfth months, with 11 deaths. The cases which reacted more favorably to the antibiotic were those occurring in children and young adults less than twenty years of age, with 2 recoveries of 12 treated in the first group and 10 recoveries among 19 in the second group. Patients suffering from bone tuberculosis also responded well to streptomycin therapy. No explanation for the great difference observed between the results obtained by the same type of therapy in the two groups can be given. Laboratory observations on sputum, cerebrospinal fluid and/or necropsy specimens obtained from 18 patients of both groups seem to belie the importance of bacterial resistance to streptomycin as a factor limiting its favorable effects in the therapy of meningitis. On the other hand, the use of the antibiotic favors the chronic evolution of patients who would otherwise have died earlier and thus permits indirectly the occurrence of meningeal complications. Furthermore, the antibiotic may increase the mobilization of toxic substances from active pulmonary lesions and cause changes in the allergic state which may also be responsible for the development of a specific

meningitis.—*Considerazioni sulla meningite tubercolare insorta in tubercolotici pretrattati con streptomicina, D. Oricchio & S. Savarino, Lotta contro tuberc., November–December, 1950, 20: 904.*—(*A. Mochi*)

Streptomycin for Minimal Tuberculosis.—In spite of apparent initial clearing of the early minimal lesion with streptomycin, tuberculosis may subsequently continue its progressive course. More and more often patients are observed who were treated with streptomycin for discrete, apparently benign lesions and who develop a "bacteriological relapse" after several months. In 10 cases of minimal nodular infiltrations, streptomycin had been administered in doses of 30 to 120 gm. and more. Regardless of whether cultures and gastrics had been positive or negative before therapy, positive bacteriologic results occurred after an interval of three months to a year without clinical or roentgenologic changes. It would appear that streptomycin is effective in clearing all congestive reactions around the tuberculous nodule but that the caseous focus itself persists and may give rise to further progression of disease if the organism is unable to promote scarring. Streptomycin can give the illusion of improvement which is, however, fragile and temporary unless it is accompanied by prolonged rest and a progressive immunization of the organism. The idea of being able to inhibit the course of tuberculosis in its beginning with streptomycin therapy has been disproved in numerous cases.—*Infiltrats discrèts pauci-bacillaires récidivants après streptomycine: Continuation de l'évolution cyclique de la tuberculose, P. Lefevre & D. Peltier, Rev. de la tuberc., 1951, 15: 259.*—(*V. Leites*)

Genitourinary Tuberculosis.—A study of the effects of the newer drugs in the treatment of genitourinary tuberculosis was carried out. The treatment regimens were: (*a*) streptomycin, 0.5 gm. twice daily up to 90 gm.; (*b*) streptomycin plus 15 gm. of *p*-aminosalicylic acid (PAS) daily; (*c*) alternate monthly courses of streptomycin and PAS for six months; (*d*) as in group 3, streptomycin plus amithiozone 50 mg. daily gradually increased to 200 mg. The cases treated were grouped as follows: (*1*) early unilateral lesion of the renal parenchyma, (*2*) unilateral renal lesion plus cystitis and nephrectomy, (*3*) bilateral renal lesion with unilateral nephrectomy, (*4*) cystitis and involvement of the remaining kidney after nephrectomy, and (*5*) very severe bilateral disease. Regimens (*c*) and (*d*) produced the best results. With treatment, the prognosis was good in patient groups (*1*) to (*3*), and poor in (*4*) and (*5*). Group (*1*) responded as well to streptomycin alone as to combinations. Group (*2*) responded best to regimens (*c*) and (*d*), if it responded at all. Group (*3*) responded best to regimens (*c*) and (*d*). In groups (*4*) and (*5*), the results were very disappointing. If hydronephrosis was present, the result was poor and a ureteral transplant was necessary. Sterilization of the urine occurred as follows: regimen (*a*), 13 of 18 cases; regimen (*b*), 4 of 7 cases; regimen (*c*), 19 of 20 cases; regimen (*d*), 6 of 6 cases. The pyelograms were either unaltered or worse in most cases. Nephrectomy was always done with streptomycin coverage; all wounds healed. It was concluded that the newer drugs are a useful adjuvant but not a substitute for surgery. Moreover, the treatment should be part of a period of sanatorium care.—*The bacteriostatic effects of the newer drugs in the treatment of genitourinary tuberculosis, J. C. Ross, J. G. Gow, & C. A. St. Hill, Lancet, May 12, 1951, 1: 1033.*—(*A. G. Cohen*)

Streptomycin for Tuberculous Pericarditis.—The manifestations of tuberculous pericarditis in 71 previously reported cases not receiving streptomycin were compared with those in 5 other patients treated with streptomycin. Symptoms of cardiac compression sometimes were not present at any time; several patients passed through the acute state of the disease with only mild systemic complaints. The important prognostic factors were: (*1*) the extent of tuberculosis elsewhere in the body and (*2*) the degree of cardiac constriction. In the group of 71 untreated

patients, 40.5 per cent died of widespread tuberculosis and 8.5 per cent died of cardiac failure; 18 patients could not be traced; 14 (19.5 per cent) survived for five years or longer and were well. One patient progressed from the acute effusive stage to that of pericardial calcification, with constrictive pericarditis twenty-two years later. A pericardectomy then relieved him of all symptoms. Control of widespread tuberculosis with streptomycin, pericardiocentesis for acute cardiac tamponade, and pericardectomy for constrictive pericarditis were the most important therapeutic measures. All of the 5 treated patients received streptomycin intramuscularly and in one it was also injected into the pericardial space, with dramatic results. In 3 a pericardectomy was done with benefit. The series demonstrated that streptomycin therapy should be started early in suspected cases of tuberculous pericarditis. The beneficial effect of prolonged bed rest and streptomycin after surgery should not be overlooked even though the symptoms have been relieved by operation.—*Streptomycin in the treatment of tuberculous pericarditis, D. Carroll, Bull. Johns Hopkins Hosp., May, 1951, 88: 425.*—(*J. S. Woolley*)

Intrapleural Streptomycin Therapy.—In children there is a vast lymphatic and vascular network just under the visceral pleura. An antibiotic injected into the pleural space is rapidly absorbed by this network and may be carried directly to parenchymal and lymphoglandular lesions in a high concentration. Twenty patients, aged two to thirteen years, were thus treated with doses of 0.1 to 0.2 gm. of streptomycin in a 1.0 cc. of solution injected intrapleurally every second day. The treatment was continued for two and one-half to five months, with a total dosage of streptomycin between 4.5 and 11.8 gm. No other treatment was used during the experiment except that 2 patients also received streptomycin intramuscularly. In order to ensure correct injection of the antibiotic between the pleurae, a small amount of air was injected and the pressure measured each time. This caused a small, hypotensive pneumothorax, not demonstrable roentgenologically. It was always kept at a minimum and could hardly have influenced the clinical course of the disease. Of the 20 patients, 16 had various types of infiltration, mostly perihilar; 2 had cavities; one had a miliary and one a nodular dissemination. Improvement was observed in 16 cases, 2 of them were clinically healed, 11 showed good results, and 3 slight improvement. Two cases did not show any change, and in one case there was some progression of the disease. One patient died and necropsy established the cause of death to be suffocation from perforation of a caseous gland in the left main bronchus. Disseminated nodular lesions, however, showed marked fibrosis. As a rule the treatment was well tolerated, but in one case a slight pleural exudation was observed and, in another, treatment had to be interrupted because of intercurrent pleurisy. The best results were obtained in the more severe cases, such as miliary or nodular dissemination.—*Sul trattamento della tubercolosi infantile con streptomicina "endopleurica", E. Rucci, Gior. ital. tuberc., November–December, 1950, 4: 456.*—(*A. Mochi*)

Clinical Streptomycin Dependence.—Progression of the disease took place in 5 patients with pulmonary tuberculosis during streptomycin therapy. In 4 of these the tubercle bacilli were found to be streptomycin dependent *in vitro*, growing poorly and slowly on ordinary media but much more rapidly when 10 mg. of streptomycin were present per cc. of medium. The strain from one patient was totally streptomycin dependent and could only be isolated on Youmans' medium containing streptomycin. The simultaneous worsening of the clinical and roentgenologic picture and the bacteriologic changes probably were not coincidences. In 4 of the 5 patients there were two distinct phases in the effect of streptomycin which was at first definitely successful and then harmful, with the concomitant appearance of streptomycin-dependent strains *in vitro*. In all cases but one,

the patients had received previous courses of streptomycin without PAS. All had cavitary disease.—*Streptomycino-dépendance et tuberculoses tertiaires aggravées par la streptomycine, A. Dufourt, J. Brun, J. Viallier, & J. C. Kalb, Rev. de la tuberc., 1951, 15: 1.—(V. Leites)*

Infection with Streptomycin-Resistant Bacilli.—A 30-year-old female lived in close contact with a patient with far advanced pulmonary tuberculosis. The latter had received a large amount of streptomycin and had streptomycin-resistant organisms. The contact was found to have bilateral cavitary tuberculosis less than one year after a negative routine roentgenogram. Her tubercle bacilli were initially resistant to more than 100 γ of streptomycin per cc.—*Réinfection exogène à bacilles streptomycino-résistants, A. Meyer, P. Naveau, & M. Naveau, Rev. de la tuberc., 1951, 15: 258.—(V. Leites)*

Streptomycin Resistance.—(*1*) The streptomycin resistance of tubercle bacilli isolated from different lesions in 24 lung specimens from tuberculous patients was determined. Strains isolated from different lesions of the same patient had the same resistance in 8 cases; in 5 cases the levels of resistance differed slightly but not significantly; in 11 cases there were real differences in the sensitivity of the examined strains. In 7 of these 11 cases there was coexistence of lesions with sensitive strains and lesions with resistant strains, the resistance exceeding 1,000 γ of streptomycin per cc. in 5 cases; in 4 cases there was coexistence of lesions with slightly resistant and highly resistant bacilli. (*2*) Strains of different sensitivity were most often found in patients who had not been treated for long periods of time and whose treatment had taken place recently. (*3*) The frequency and degree of resistance was in direct proportion to the bacillary content of the lesions from which they were isolated. Thus, lesions yielding less than 100 colonies per 100 mg. of tissue showed 48.5 per cent sensitive strains; 25.8 per cent, moderately resistant strains; and 25.8 per cent, highly resistant strains. For lesions yielding more than 1,000 colonies the corresponding figures were 10.9 per cent, 21.7 per cent, and 67.4 per cent. (*4*) Lesions with caseous liquefaction more often yielded resistant strains than did lesions with solid caseation. The former were much richer in bacilli than the latter. (Authors' summary).—*La streptomycino-résistance de souches de bacilles de Koch provenant de lésions pulmonaires différentes d'un meme malade, G. Canetti & A. Saenz, Ann. Inst. Pasteur, March, 1951, 80: 258.—(V. Leites)*

Pathology in Streptomycin-Treated Tuberculosis.—The findings in an autopsy series of 34 cases emphasize the importance of knowing the relative streptomycin sensitivity of the tubercle bacilli present. During the period of maximal streptomycin effect the healing process is characterized by the more rapid healing of foci, the lack of perifocal reaction about the pulmonary foci, and the absence of laryngeal and intestinal invasion. The onset of resistance is marked by the appearance of new foci similar to those in untreated cases; intestinal and laryngeal tuberculosis again develops. There were 20 such cases in this series; 15 exhibited intestinal, and 11 laryngeal, lesions. Such lesions were entirely absent in the 7 cases known to be streptomycin sensitive from 15 to 97 days before death. In one case of coexisting meningeal and pulmonary disease the organisms recovered from the spinal fluid were sensitive while those from the lungs were not.—*Pathology of tuberculosis treated with streptomycin: A review of 34 cases, O. Auerbach, L. Weiss, & G. N. Stemmermann, Dis. of Chest, February, 1951, 19: 145.—(E. A. Rouff)*

Streptomycin and Eosinophilia.—The effect of streptomycin treatment on the percentage of eosinophils in the blood was studied in 26 patients suffering from miliary tuberculosis and tuberculous meningitis. The percentage varied in these patients from 3 to 35 per cent. Factors responsible for such variations

may have been the patient's condition, the dosage of streptomycin, the duration of treatment, and the location of the tuberculous lesions. In some persons the tendency toward marked eosinophilia was apparently a personal characteristic although it has not been proved that allergy is always the underlying factor in such cases. As a rule, the eosinophilia was greatest when the patient had received between 100 and 200 gm. of streptomycin, after which it usually diminished. It was not likely to return to a normal percentage until the treatment had been discontinued. Increase in the eosinophil percentage was not a constant association of cochleo-vestibular complications nor did it appear to have prognostic significance. Blood eosinophilia is believed to be usual in patients with hematogenous tuberculosis treated with streptomycin and is doubtless due to the effect of the substance on the patient's organism. It has no definite significance so far as has yet been determined. —*L'éosinophile sanguine chez les sujets atteints de miliaires ou de méningites tuberculeuses et traités par la streptomycine, J. Tapie, Laporte, Monnier, Gontier, & Turnin, Poumon, April, 1951, 7: 189.*—(*A. T. Laird*)

Streptomycin-*p*-Aminosalicylate.—The antituberculous effect of streptomycin—*p*-aminosalicylate containing 1 gm. of streptomycin base in each 1.79 gm. was studied *in vitro* and *in vivo*. *In vitro* experiments with the human tubercle bacilli strain H512 on Dubos medium and with the strain of Arloing and Courmont showed that the tuberculostatic effect of streptomycin—*p*-aminosalicylate was slightly inferior to that of an equivalent mixture of streptomycin and PAS. Three groups of mice were infected with 1 mg. of H512. The first group (untreated) served as controls. The second group received 1.8 mg. of streptomycin-PAS daily. The third group was treated with 0.8 mg. of PAS and 1,000 units of streptomycin daily. The mortality in the untreated control group was 100 per cent, death occurring after 19 to 40 days. Pulmonary lesions were very severe and no normal lungs were found. Mice treated with the experimental drug had a mortality of 10 per cent, death occurring after 27 to 28 days; 90 per cent survived for 53 days, at which time they were sacrificed. The pulmonary lesions were generally slight. Normal lungs were present in 66 per cent of the animals. Mice treated with streptomycin and PAS had a mortality of 5.2 per cent (dying after 39 days); 94.8 per cent survived for 48 to 55 days when lesions similar to those in the second group were noted; 68 per cent of the animals had normal lungs. The *in vivo* effects of the experimental compound and of PAS and streptomycin given separately are thus essentially the same. The only advantage seems to be the simultaneous administration.—*Effets anti-tuberculeux du p-aminosalicylate de streptomycine, C. Levaditi, A. Vaisman, H. Chaigneau, & J. Henry-Eveno, Ann. Inst. Pasteur, December, 1950, 79: 886.*—(*V. Leites*)

PAS.—*p*-Aminosalicylic acid (PAS) was administered in large doses to 121 patients for fifty days to eight months: they were observed after treatment for three to six months. Cavitary cases eligible for pneumothorax therapy and cases in such danger as to necessitate streptomycin were eliminated. The indications for PAS were: (*1*) types of tuberculosis in which spontaneous regression was probable but a more rapid improvement was expected with PAS; (*2*) cases of probably incurable phthisis; (*3*) cases of tuberculosis resistant to streptomycin; (*4*) cases in which PAS was used as an adjuvant to collapse therapy. The average daily dose was 20 gm. Often 25 gm. were tolerated and 30 gm. were given to patients weighing more than 70 kg. After forty days, PAS was interrupted for one week; sputum was then collected for smear, culture, guinea pig inoculation, and for determination of bacillary resistance to PAS *in vitro*. This method greatly increased the number of positive sputum results. Intolerance to the drug developed in 12 per cent of patients within the first two weeks. Of 121 patients, 8 had to reduce their dose to 12 gm.; 7 per cent showed intolerance after two months of treatment. No serious toxic

manifestations were observed. Fever was favorably influenced in 65 per cent of the cases. There was gain in weight in 15 per cent, loss of weight in 12 per cent, and an unchanged weight in 73 per cent of the cases. In the cases where clinical improvement occurred, it was observed within the first two to three weeks and was independent of any anatomic improvement. Diminution of expectoration occurred in 52 per cent of cases and was often immediate; in spite of continuation of treatment, this effect wore off after two to three months. Of 43 cavitary cases, 60 per cent retained positive sputum after four months, 57 per cent after five months. The effect on roentgenographic changes was generally slight; there were 17.8 per cent substantial results and 32.6 per cent improvements, frequently of a transient nature. PAS gave the best results in recent nodular infiltrations without cavitation or with only small "highlights," 56 per cent showing considerable roentgenographic clearing. Of 60 patients with cavitary lesions, only 3.3 per cent showed closure during PAS therapy. In 30 per cent there was diminution in the diameter of cavities, 12 per cent increasing subsequently. In streptomycin-resistant patients, PAS gave an unexpectedly high number of good results. In the nodular forms there were 78 per cent of improvements. The effect of PAS on pleural effusions and empyema was unremarkable.—*Le P.A.S. dans le traitement de la tuberculose pulmonaire non miliaire, P. Veran & U. Guinot, Rev. de la tuberc., 1951, 15: 73.*—(*V. Leites*)

Drug Fever with PAS.—Drug fever is considered a rare toxic reaction to *p*-aminosalicylic acid (PAS). Only one instance was noted in two years' experience and this was in an 11-year-old Chinese boy who received 6 gm. of sodium PAS daily. His temperature had been normal for several days but on the eighth day of administration of the drug it rose to 38.8°, to 39.5°C. on the following day, and to 40°C. on the tenth day. At this time the patient also noted severe headache, general discomfort, nausea, and vomiting. An erythematous rash was noted over the entire body. Administration of the drug was stopped at this time, and all of the symptoms began to disappear on the following day, the temperature dropping to normal. Two weeks later the patient was given another single dose of the same brand of medication, the dose being 1.5 gm. Two hours later tachycardia, headache, redness of the conjunctiva, general discomfort, and malaise were noted. Several hours following this a general erythematous rash appeared over the entire body, especially on the face and trunk, and his body temperature rose to 39.4°C. He was entirely well on the following day with no further medication. On questioning the patient, it was found that he previously had been shown to be sensitive to streptomycin and had had skin rash and fever.—*Drug fever following the use of para-aminosalicylic acid: A case report, B. H. Y. T'ang, Chinese M. J., March–April, 1951, 69: 185.*—(*L. Hyde*)

PAS Serum Concentrations.—Free and total *p*-aminosalicylic acid (PAS) was determined in the blood serum of 26 patients after single and multiple oral doses of various forms of PAS and the sodium salt of PAS. PAS powder and the sodium salt yielded approximately the same concentrations after single or multiple oral doses as measured an hour and a half after administration. Compressed tablets or enteric-coated granules of PAS gave lower concentrations than either of the first two, but this is believed to be due more to delayed than to incomplete absorption. Sodium bicarbonate or ammonium chloride had practically no effect on the serum concentrations. Most patients have been able to take a larger daily dose of PAS in the form of the sodium salt than when any other preparation was given.—*Serum concentrations of para-aminosalicylic acid (PAS) produced by various forms of PAS, D. K. Duncan, D. T. Carr, K. H. Pfuetze, & M. H. Power, Dis. of Chest, February, 1951, 19: 138.*—(*E. A. Rouff*)

Amithiozone for Pulmonary Tuberculosis.—Amithiozone (TBI/698) was given to 49

patients with pulmonary tuberculosis. The maximal daily dose was 300 mg. but most received only 200 mg. daily. Therapy usually was started with 50 mg. twice daily and the dose was increased progressively by 25 mg. every week. The total amount administered ranged from 2 to 3 gm. Gastrointestinal disturbances occurred in 8 patients, urticaria in 2; and conjunctivitis in 3 patients, one of whom had to discontinue treatment. Urinalyses remained negative but most patients showed a diminution in the number of leukocytes, the blood picture being otherwise unremarkable. Of 6 patients with abundant expectoration, there was disappearance of sputum in 4. In one of 2 febrile patients, defervescence began after six days of treatment. The body weight was not appreciably modified. Two cavities of long standing were not influenced; of 10 patients with fresh cavities, the results were "favorable" in 6, "uncertain" in 3, and undetectable in one case. Among 39 cases of so-called "nodular tuberculosis", there were 13 good results and 11 improvements.—*Quarante-neuf cas de tuberculose pulmonaire traités par la thiosémicarbazone (TB 1), Joussaume, Morand, & Pivetaud, Rev. de la tubere., 1951, 15: 283.—(V. Leites)*

Amithiozone for Tuberculosis.—Amithiozone (TB I) was given to 26 patients with pulmonary tuberculosis. Of these, 4 had good results with almost complete roentgenographic clearing and conversion of sputum, 8 improved with considerable roentgenographic clearing, and 14 remained unimproved. Thus, the roentgenogram was favorably influenced in approximately half of the cases. Best results were observed in disease of recent origin; no effect was noted in pleural complications. Constitutional symptoms were favorably influenced with gain in weight, defervescence, and diminution of cough and expectoration. Slight anemia was observed in 25 per cent of the cases a few weeks after the onset of treatment.—*Le traitement de la tuberculose pulmonaire et pleurale par le thiosémicarbazone (TB 1/698): Premiers resultats, J. Catta, Semaine d. hôp. Paris, May 10, 1951, 27: 1639.—(V. Leites)*

Pulmonary Excretion of Penicillin.—Ordinary penicillin is not excreted by the lungs. A study was made of benzylpenicillin diethylamino ester hydriodide (L.G.1) and its hydrochloride (L.G.2) which is more soluble in water. One million units of L.G.1 or L.G.2 were given intramuscularly every twelve hours for one week. The findings were compared with those obtained with identical doses of procaine penicillin or sodium penicillin. All of the patients selected had copious sputum. L.G.1 was present in the sputum within six hours after injection in concentrations greater than ordinary penicillin. L.G.2 produced a similar effect but disappeared more rapidly. There was great variation among patients. In twenty-four-hour specimens there were substantial amounts of L.G.1 and L.G.2. No toxic effects were noted. The volume of sputum was reduced and the patients felt better.—*Concentration of penicillin in the lungs, A. G. S. Heathcote & E. Nassau, Lancet, June 9, 1951, 1: 1255.—(A. G. Cohen)*

Bacitracin for Pneumonia.—Bacitracin, in doses of 30,000 to 99,000 units intramuscularly every six hours, was given to 14 patients with lobar pneumonia. Twelve recovered, one had to be treated with penicillin, and one died of bacterial endocarditis although his pneumonia responded well to treatment. All of the patients exhibited moderate albuminuria and casts and had reduced concentrative power of the kidney and phenolsulfonphthalein excretion lasting for a variable time after therapy. No elevation of nonprotein-nitrogen was observed. At present, the nephrotoxicity of bacitracin precludes its use in preference to penicillin. This should not cause hesitation in cases where there is a sensitive organism that did not respond to penicillin or other therapy. Its use is not recommended in cases with pre-existing parenchymatous renal disease but toxic albuminuria is not considered a contraindication. None of the patients in

this series showed any permanent or serious damage to the kidneys and the serial studies in mice show that nonlethal doses of bacitracin leave no permanent scars in the kidneys.—*The treatment of pneumonia with bacitracin, E. H. Reimer, Jr., F. N. Bailey, & E. Appelbaum, Ann. Int. Med., May, 1951, 34: 1232.*—(*T. H. Noehren*)

LABORATORY STUDIES

Variability of BCG Strains.—The morphologic growth pattern of mammalian tubercle bacilli seems to be correlated with their virulence, virulent strains tending to form "cords" in liquid media and spreading colonies on the surface of agar media. Certain strains show no such orientation and are devoid of virulence. The morphology of attenuated BCG is less defined, probably because the various cultures of BCG are heterogeneous with reference to their colonial morphology; they also differ in their ability to cause lesions in experimental animals. Three strains of BCG were propagated for two years in a liquid medium containing Tween 80, 0.02 per cent, and albumin, 0.5 per cent. A fourth strain was less completely investigated. Dilute subcultures were planted on the surface of agar containing oleic acid, albumin, and 0.25 per cent glucose. The colonies as seen with the microscope were classified as follows: (*1*) spreading with a dense opaque center surrounded by a halo of serpentine strands ("cords") folded close together; (*2*) intermediate with a larger dark center and smaller halo in which the serpentine pattern could be seen; (*3*) nonspreading with greater opacity and lack of orientation. Intermediate colonies predominated (75 to 85 per cent) in two strains. In the other two strains, 80 per cent of the colonies were spreading in type with the formation of tight cords. Spreading and nonspreading colonies from the parent cultures were reinoculated and tended to retain their characteristics although completely homogeneous substrains of pure nonspreading colonies could not be obtained. Thus, the BCG cultures were made up of a morphologically heterogeneous population. Although the relative proportion of the colonial types could be changed, the parent cultures of BCG remain fairly constant during repeated transfers *in vitro* and during passages in mice and guinea pigs. Cultures recovered from these animals generally consisted of the same proportion of the three colonial types which was present in the inoculating cultures, regardless of the time which elapsed between infection and recovery. The three strains tested differed widely in their ability to produce pathologic lesions. Two caused macroscopic lesions; the third induced no macroscopic lesions in guinea pigs or mice, except at the site of the intracutaneous injection in guinea pigs. The three strains were cultivated under identical conditions and their differences in pathogenic properties must be due to intrinsic characteristics rather than to environmental factors. The spreading and nonspreading substrains exhibited the same pathogenic potency as their parent strains. It seems, therefore, that the ability of the BCG cultures to multiply or survive in tissue is conditioned by factors independent of those manifested in the tendency to form cords or spreading colonies.—*Variability of BCG strains (Bacillus Calmette-Guerin), W. E. Suter & R. J. Dubos, J. Exper. Med., June 1, 1951, 93: 559.*—(*J. S. Woolley*)

BCG in the Hamster.—The hamster (*Mesocricetus auratus*) is sensitive to tuberculosis. Human and bovine tubercle bacilli produce the same type of lesions in the hamster as in the guinea pig. BCG was found to produce similar or identical pathologic changes in lungs, liver, spleen, lymph nodes, the fibrous capsule of the kidney, and the peritoneum. In the liver there was first an infiltration of leukocytes, then of macrophages; epithelioid cells and Langhans' cells developed later. Often the foci do not contain a necrotic center but this was lacking also in some lesions produced by tubercle bacilli. Subsequently a fibrous capsule is formed around the lesion. In some animals the liver and spleen showed amyloid deposits.—*Étude anatomo-pathologique des lésions du hamster inoculé avec le bacille BCG, J. L. Nicod, P. Haudurov, & W.*

Rosset, Presse méd., May 5, 1951: 605.—(V. Leites)

Hamsters for Differentiating Tubercle Bacilli Types.—Rabbits have long been used to differentiate the bovine type of tubercle bacilli which is more virulent than the human type for the rabbit. The bovine type is also more virulent for the field vole, but previous work using the Chinese hamster has indicated that the human type is more virulent in this latter animal. Eighty hamsters were infected with five strains of *M. tuberculosis* belonging to either human or bovine types. The hamsters were divided into six groups and were injected with different types of tubercle bacilli and by different routes. On the whole, the bovine strains were more virulent to hamsters than the human strains and with either strain the intratesticular and intraperitoneal routes produced a more acute disease than the subcutaneous route of injection. The average survival time after injection of a bovine type was between 18 and 30 days; using the human type H37Rv strain of tubercle bacilli, it was between 86 and 94 days. Another human strain produced no death in 35 days. If these preliminary results are confirmed by more extensive experimentation, the Chinese hamsters may aid in differentiation of human and bovine types of *M. tuberculosis.—The use of the Chinese hamsters (Cricetulus griseus) in the study of Mycobacterium tuberculosis: 2. For the differentiation of human and bovine types, F. L. Wang, Chinese M. J., March-April, 1951, 69: 155.—(L. Hyde)*

Bovine Bacilli.—There were almost no circulating leukocytes with abnormal granulations in guinea pigs infected with bovine or avian bacilli in contrast to their presence after these animals were injected with serum from tuberculous patients or with cultures of human tubercle bacilli. This fact can be utilized for diagnostic purposes. If guinea pigs injected with a culture of tubercle bacilli or a tuberculous pathologic specimen do not show an increase in abnormally granulated leukocytes after the usual interval, one may exclude the presence of human tubercle bacilli and assume that one is dealing with a bovine strain. Another differential point can be seen in slide cultures on Youmans' medium and in slides of emulsified colonies from the Löwenstein medium. Bovine bacilli are arranged in oblong and sinuous formations called "moustache" forms. These are not seen with human-type bacilli.—*Recherches expérimentales sur le bacilli bovin: Différenciation pratique du bacille bovin, R. Benda & D. A. Urquia, Semaine d. hôp. Paris, May 30, 1951, 27: 1730.—(V. Leites)*

Hemagglutination Test Evaluation.—A clinical evaluation was made of the Scott and Smith modification of the hemagglutination test for tuberculosis in normal individuals and in subjects with tuberculosis, sarcoidosis, following BCG vaccination, and with miscellaneous diseases. Tests were performed on sera obtained from more than 400 patients. Sera of normal individuals usually gave no titer or low titers but a few had high titers. The tuberculin-positive group had somewhat higher titers than the tuberculin-negative group. In general, those groups with active tuberculosis had higher titers than those with inactive disease but extremes of titers were found in both groups. The average titers in active minimal and in moderately advanced tuberculosis were significantly higher than that of the normal group but many patients in the minimal group had low titers. The highest average titer was in the group with far advanced tuberculosis. In a small group of patients with extrapulmonary tuberculosis the average titer was low and the range of titers was similar to that of the normal groups. In a small group of patients with tuberculosis followed over a long period, some showed elevation of titers, some showed depression, and others remained the same. There was no definite correlation of the effect of treatment with the trends of titers. Sera of 22 patients with proved sarcoidosis yielded titers which were similar to the normal groups in their distribution and in their low average. Following BCG vaccination of tuberculin-negative individuals

there was an inconsistent response. Only 25 per cent showed a rise in titer although all cases converted to tuberculin positivity. Patients with syphilis, various pulmonary diseases, and other miscellaneous diseases gave no indication of "false positives." The range of titers was similar to that of the normal groups. With the Old Tuberculin antigen as used here, the hemagglutination test for tuberculosis is by itself of limited clinical value. Elevation of titer is suggestive of tuberculosis in cases in which the differential diagnosis includes this disease. Absence of demonstrable circulating agglutinins does not rule out the presence of active tuberculosis (Authors' summary).—*An evaluation of the hemagglutination test for tuberculosis, J. W. Fleming, E. H. Runyon, & M. M. Cummings, Am. J. Med., June, 1951, 10: 704.—(T. H. Noehren)*

Complement Fixation and Tuberculosis.—Sheep red cells coated with an extract of the phenol-insoluble residue of tubercle bacilli, when suspended with anti-BCG rabbit serum in the presence of guinea pig complement, will lyse. In the one serum tested, the lysis was present in much greater dilutions of serum than was hemagglutination, as tested by the method of Middlebrook and Dubos. That the phenomenon is probably an antigen-antibody reaction is suggested by the fact that the addition of the extract to the serum will absorb out the active fraction and abolish the reaction causing lysis. Speculation is possible concerning the use of this as a diagnostic test in tuberculosis.—*The lysis by complement of sheep erythrocytes sensitized with an extract of Mycobacterium tuberculosis and anti-BCG rabbit serum, S. Fisher, Australian J. Exper. Biol. & M. Sc., November, 1950, 28: 613.—(S. Hadley)*

Antibody in Tuberculosis.—The author describes a modification of his test for antibody in the serum of tuberculous patients. Human erythrocytes treated with extracts or products of culture filtrates of tubercle bacilli will agglutinate if antibody-containing serum is added. If fresh guinea pig complement is also added, hemolysis may occur. Since there may be a wide difference in the capacity of a single serum to demonstrate these two phenomena, there is evidence that these two tests probably do not measure the same antibodies.—*A hemolytic modification of the hemagglutination test for antibodies against tubercle bacillus antigens, G. Middlebrook, J. Clin. Investigation, November, 1950, 29: 1480.—(S. Hadley)*

Electrocardiograms and Pneumoperitoneum.—Standard and unipolar lead electrocardiograms were taken in both the recumbent and sitting positions on 19 patients receiving pneumoperitoneum therapy for pulmonary tuberculosis. In the recumbent position, the standard lead electrocardiograms were normal without axis deviation in the majority of patients. This is in contrast to some earlier reports. With the patients in the sitting position, however, most of the patients showed a left axis shift by the standard lead method and frequently had a deep Q_3. The same patients had evidence of a more horizontal position of the heart in unipolar electrocardiograms taken in the sitting position. These findings are in contrast to the electrocardiographic changes in normal individuals upon arising from the recumbent to the sitting position. Fluoroscopic examinations in both positions and measurements of the diaphragmatic changes failed to demonstrate any significant rise of the hemidiaphragm on sitting up, thus showing that the presence of left axis shift is not due to rise of the diaphragm with resultant elevation of the apex of the heart; rather, it occurs in spite of descent of the diaphragm and depression of the apex. This substantiates the theory that rotation of the heart rather than upward displacement is the cause of the left axis shift.—*The effect of pneumoperitoneum on the electrocardiogram, A. Pollak, Dis. of Chest, January, 1951, 19: 56.—(E. A. Rouff)*

Cortisone in Bacterial Diseases.—Cortisone has the ability to alleviate symptoms despite bacteriologic evidence of continued disease.

In rats given cortisone five days before being experimentally infected with streptococcal and pneumococcal pneumonia, the survival rates were lower than in the control group. Copious edema fluid accumulated as a result of the infection but it was relatively acellular. Despite the absence of a growth-stimulating effect of bacteria, the number of organisms present in the experimental lesions in the treated animals was much larger than in the control.—*The effect of cortisone on acute bacterial infections, R. Glaser, J. Berry, L. Loeb, & W. Wood, J. Clin. Investigation, June, 1951, 30: 640.*—(*S. Hadley*)

Effect of Posture on the Dead Space.—The effect of changes in posture on functional residual capacity and respiratory dead space were measured in 5 male subjects. All of the subjects had smaller functional residual capacities in the semi-reclining and supine positions than when sitting. The average decreases were 556 and 787 ml., respectively. The average dead space-tidal volume fraction and the respiratory dead space were similarly greater when the subjects were sitting than when supine.—*Lung function studies: IV. Postural changes in respiratory dead space and functional residual capacity, W. Fowler, J. Clin. Investigation, November, 1950, 29: 1457.*—(*S. Hadley*)

Dead Space in Emphysema.—Continuous measurements were made of the nitrogen concentration and the volume-flow of gas expired after oxygen and room-air inhalation in 10 normal young men, 11 normal old men, and 12 elderly male patients with marked emphysema. Calculations of the respiratory dead space, using Bohr's formula, and of the tidal volume showed the respiratory dead space after maximal inspiration to be similar in all of the three groups. After quiet breathing the space was significantly greater in the emphysematous group. The tidal volume, however, decreases in the emphysematous patients after breathing oxygen. Measurements of the dead space were made in pulmonary emphysema by a new technique, older methods having presented difficulty. It is suggested that the increase in dead space in emphysema may result from a loss of elasticity of the bronchial tree as well as of the lung as a whole.—*Lung function studies: V. Respiratory dead space in old age and in pulmonary emphysema, W. Fowler, J. Clin. Investigation, November, 1950, 29: 1439.*—(*S. Hadley*)

Lung Volume in Tobacco Users.—The total lung volume and its subdivisions were studied in 58 healthy men. The findings were correlated with the use of tobacco. Smoking caused a slight decrease in vital capacity and a greater increase in residual air and in the ratio of residual air to total lung volume. The changes were more pronounced when the recent consumption of tobacco was high. Smoking also produced a diminution in chest expansion.—*The effect of tobacco on lung volume, A. G. W. Whitfield, W. M. Arnott, & H. A. H. Waterhouse, Quart. J. Med., April, 1951, 20: 141.*—(*A. G. Cohen*)

Cell Changes with Streptodornase.—Detailed studies of the effect of the streptococcal enzymes on the cellular components in empyema fluid and other purulent exudates were carried out, using supravital staining in association with the usual total and differential cell counts. From these it was clearly shown that the local introduction of active enzymes only is followed by a considerable outpouring of living, motile, phagocytosing polymorphonuclear leukocytes, which reaches a maximum in twenty-four hours. This is accompanied by a marked fall in the number of degenerating cells. Definite evidence is presented to show that streptodornase does not injure the living, motile, phagocytosing cell. Depolymerization of the desoxyribonucleic acid by the streptococcal desoxyribonuclease not only liquefies the highly viscous purulent material but also causes a breaking up of the clumped leukocytes. This will account, in part, for the great increase in cells useful for combatting the infection.—*Cytological studies in association with local injections of streptokinase-streptodornase into patients, A. Johnson, J. Clin. Investigation, October, 1950, 29: 1376.*—(*S. Hadley*)

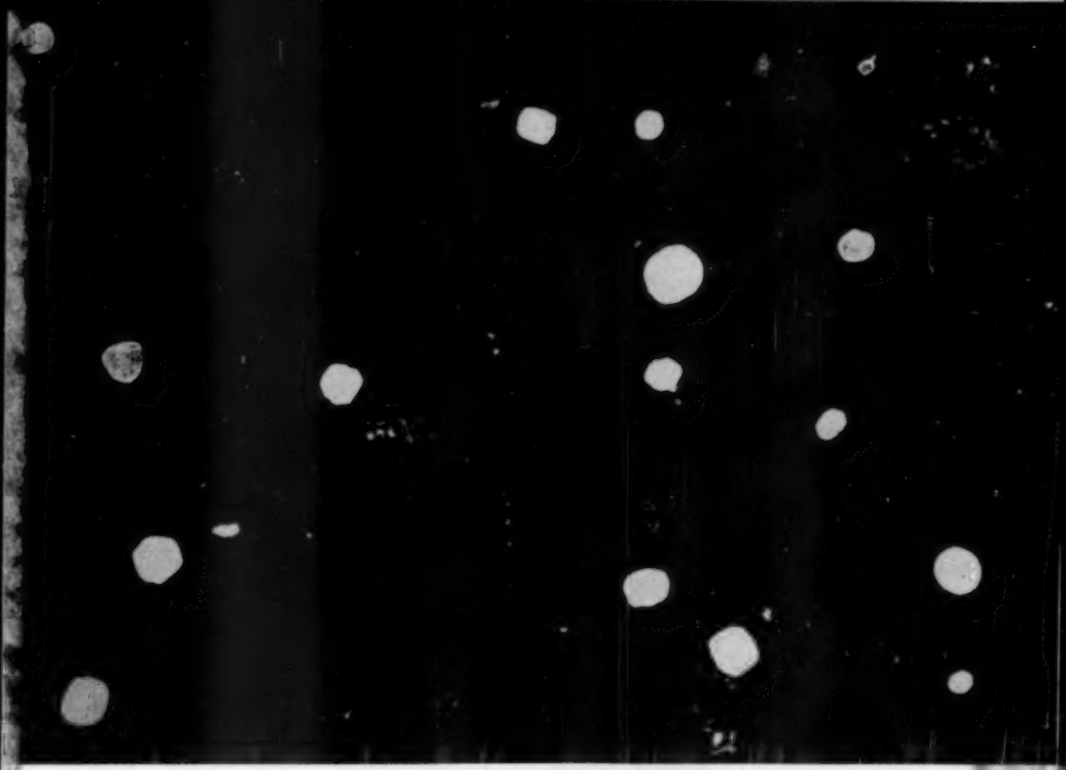
PUBLIC HEALTH AND EPIDEMIOLOGY

Tuberculous Infection and Morbidity.—A series of 9,000 persons of both sexes and of all ages was divided at the beginning of the observation period into tuberculin-positive and tuberculin-negative groups, as tested by the Pirquet method. None had evidence of tuberculosis. The follow-up has been pursued continuously for twenty-five years, the observation periods ranging from one to twenty-eight years and the average being eleven years. The total observation years approximates 100,000. The tuberculin-negative individuals were subdivided into two groups: (*1*) Student nurses at the Oslo Municipal Hospital between 1924 and 1936. Their exposure was massive and continuous enough to produce a 100 per cent conversion to positive tuberculin reactions within three years, an annual increment of infection of 33 per cent. (*2*) The general population of Oslo who had minimal and sporadic exposure to tuberculosis as compared to the student nurses. In this group the annual increment of infection was only 3 per cent. These two extremes of infection, massive or slight, are correlated with subsequent tuberculosis morbidity: Of 253 student nurses who all converted during their training period 106 developed tuberculous disease (42 per cent) and 13 died (5.1 per cent) during an average observation period of nine years and a total observation period of 2,369 years. This group is compared to 948 women of approximately the same age who were initially tuberculin negative. The degree of infection here is 50 per 1,000 observation years; 250 women were infected during this period; 51 of these developed tuberculous disease (20 per cent) and 5 died (2 per cent). Morbidity and mortality were thus approximately twice as high in the group with massive and continuous exposure. A second comparison was made with 1,716 female residents of Oslo of all ages who were tuberculin negative at the onset of observation. The degree of infection is 37 per 1,000 observation years; the morbidity, 25.5 per cent; and the mortality, 2.2 per cent. In 1,415 tuberculin-negative male residents of Oslo the degree of infection was 42 per 1,000 observation years, tuberculous morbidity was 16 per cent, and mortality was 2.3 per cent. Thus, with the same slight exposure, morbidity was definitely lower in men. For the analysis of tuberculous reinfection, those persons were studied who had a positive tuberculin test at the beginning of observation. Reinfection with massive doses was studied in 668 student nurses who were tuberculin positive at the beginning of training. During their three-year course the tuberculosis morbidity was 12.4 per 1,000 observation years and the mortality was 0.6. Of these nurses, 504 remained under observation after graduation when the majority were no longer exposed to infectious cases. For a total of 5,670 observation years, the mortality was 0.2 and the morbidity was 3 per 1,000 observation years, only one-fourth of that under conditions of massive exposure. As a comparison group, 398 tuberculin-positive women between the ages of 20 and 22 from the general pouplation of Oslo were observed with a morbidity of 3.6 and a mortality of 0.4 per 1,000 years. These findings, as well as similar figures among tuberculin-positive men in this age group, confirm the difference of massive and reduced exposure in the course of tuberculous reinfection. Consequently, it is not the age which is the reason for the high morbidity among student nurses. The following figures illustrate the low general risk of tuberulosis for tuberculin-positive individuals under ordinary conditions of life: 1,551 women of all ages had a tuberculosis morbidity of 1.8 and a mortality of 0.4 per 1,000 years during a total of 19,364 observation years. Among 1,564 men the figures were the same. Familial contacts further demonstrated the influence of massive exposure on the subsequent development of tuberculosis: Of 952 tuberculin-positive Oslo residents who had cases of tuberculosis in their families, 4.8 developed tuberculosis and 1.6 died. Of 2,163 tuberculin-positive individuals without tuberculosis contact in the family, only 1.1 per cent developed tuberculosis and 0.09 per cent died. Of 617 tuberculin-negative persons with family exposure, 16 per cent developed tuberculosis and 2.3 per cent died, as compared to 6.6

per cent and 0.6 per cent in nonreactors without family contact. These figures might be interpreted as proof of the importance of familial disposition in tuberculosis and against the importance of dosage of infection. The following figures are given as a counter argument: Student nurses with and without familial exposure to tuberculosis leave their home environment at the onset of training and are all placed in the same conditions of massive exposure. For tuberculin-positive students with familial exposure, the subsequent tuberculosis morbidity was 6.1 per cent; it was 5.8 per cent for those without family exposure. Among tuberculin-negative students, the morbidity was 40 per cent for those with a family history of tuberculosis and 42 per cent for the other group. In student nurses vaccinated with BCG, the morbidity was twice as high as in vaccinated groups of the general population. It is possible that the malignant character of certain tuberculous infections is only the consequence of the frequency and intensity of infection and that rare and minimal infections constitute the best vaccination.—*Rapport existant entre l'infection et les maladies tuberculeuses, J. Heimbeck, Rev. de la tuberc., 1951, 15: 197.*—(*V. Leites*)

Routine Roentgenography in Children.—A total of 349 children aged five to fifteen years were admitted after a routine roentgenogram to a children's sanatorium in France from 1932 to 1950. During the period 1932 to 1940, cases of tuberculosis diagnosed on routine examination represented 3 per cent of all admissions. This proportion rose to 16.2 per cent between 1940 and 1945 and to 22 per cent between 1945 and 1950. The source of infection could be determined in 59.5 per cent of cases. In 73 per cent of these the infection was of familial origin. Hilar adenopathies represented 31 per cent of all routinely discovered abnormalities; 16.3 per cent were pleural forms; pulmonary involvement was present in 52.7 per cent (184 cases). Of these, 134 were unilateral; 76 showed cavitation. Among the noncavitary forms there were 79 infiltrates, 9 nodular forms, 8 lobar consolidations, 8 calcifications, 4 diffuse lesions, and 18 cases of associated bronchial disease. Of the total number of 349 routinely discovered cases, 100 (28.7 per cent) had positive sputum; 61 of these had extensive, bilateral, cavitary lesions; 80 of the 100 positive cases were completely asymptomatic. The total number of clinically latent forms was 64.3 per cent. In view of the high incidence of cases with positive sputum and cavitary tuberculosis in asymptomatic children, the importance of routine roentgenography is stressed. Preliminary screening with tuberculin is recommended.—*Étude de 349 cas de tuberculose pulmonaire entrés au Sanatorium d'enfants après dépistage systématique, P. Lowys & J. Lengrand, Rev. de la tuberc., 1951, 15: 229.*—(*V. Leites*)

Laboratory Infection with Streptomycin-Resistant Bacilli.—A 24-year-old student worked in a tuberculosis laboratory injecting streptomycin into guinea pigs which had been infected with bacilli resistant to 1,000 γ of streptomycin per cc. She also sealed previously corked culture tubes. Her tuberculin test had been found positive three years earlier when her chest roentgenograms were normal. After one year of work in the laboratory, a roentgenogram disclosed a minimal tuberculous lesion in the left lung and the gastric washings contained acid-fast bacilli. On Youmans' medium the cultures were resistant to 500 γ of streptomycin per cc. The patient had never received streptomycin, and strains of tubercle bacilli spontaneously resistant to such high concentrations are very rare. On the other hand, resistant strains were used daily in the laboratory and her infection was doubtless exogenous. The resistance of the patient's strain did not correspond to the one used for infecting the guinea pigs which she handled and she did not prepare suspensions or cultures. This case shows that in a tuberculosis laboratory even the personnel not using infectious material run a risk of contamination.—*Tuberculose de réinfection à bacilles streptomycino-résistants contractée au laboratoire, G. Canetti, A. Saenz, R. Thibier, & J. Chevassu-Perigny, Rev. de la tuberc., 1951, 15: 128.*—(*V. Leites*)



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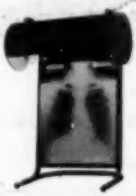

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*TEMPEL, C. W., FREDERIC, J. H. Jr., MARDIS, R. C., TOWBIN, M. N., and DYE, W. E. Combined intermittent regimens employing streptomycin and PAS. Am. Rev. Tuberc., 1951, 63, 295

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Copley Plaza	Copley Square	$5.85– $7.85	$9.90– $14.00
Copley Square	Copley Square	$4.00	$7.00– $8.80
Kenmore	Kenmore Square	$5.50– $8.50	$9.50– $12.50
Lenox	Exeter Street	$4.50– $5.50	$9.00
Manger	North Station	$4.75– $7.75	$8.00– $10.50
Parker House	School Street	$6.00– $8.00	$10.00–$14.00
Pioneer	410 Stuart Street	$3.50– $3.75	$5.50– $7.00
Shelton	91 Bay State Road	$5.00– $7.00	$8.00– $10.00
Somerset	400 Commonwealth Ave.	$7.00– $8.00	$12.00
Statler	Park Square	$6.00–$10.00	$12.00–$16.00
Touraine	62 Boylston Street	$5.00– $7.00	$9.00– $12.00
Vendome	160 Commonwealth Ave.	$4.00– $6.00	$7.00– $10.00
University Club	40 Trinity Place	$6.00	$8.00

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Hycodan® BITARTRATE

(Hydrocodone Bitartrate)

Three forms available: Oral Tablets (5 mg. per tablet), Syrup (5 mg. per teaspoonful), Powder (for compounding). May be habit forming; narcotic blank required. Average adult dose 5 mg. Literature on request.

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